THE HORMONES

PHYSIOLOGY, CHEMISTRY AND APPLICATIONS

VOLUME I

THE HORMONES

PHYSIOLOGY, CHEMISTRY AND APPLICATIONS

Edited by

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VOLUME I



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Preface

The study of hormones is one of the great unifying threads running through modern biology, extending all the way from botany through marine zoology to clinical medicine, and with the active participation of chemistry at almost every stage but the earliest For this very reason, however, the data and materials dealing with hormones have been published in widely scattered journals While some parts of the subject have already been brought together in review form, in no place has a comprehensive presentation, at the research level, been assembled It is the purpose of this treatise to bring together the great scattered mass of information covering the roles of hormones in a wide diversity of vital processes, and their pathological as well as normal physiology For this purpose the Editors have been fortunate in having the collaboration of a group of authors, each one an expert and an active investigator in his particular field The experimentalism essential to our advancing knowledge, therefore, receives full emphasis, while practical applications, particularly in applied biology and medicine, have been brought in wherever possible

The division of the treatise into two volumes is largely a matter of convenience rather than of principle The first volume contains the chemistry of the hormones, the role of hormones in organisms other than mammals, and some aspects of the animal physiology The second volume will contain the bulk of the mammalian endocrinology proper, with clinical applications

In regard to minutiae such as terminology and spelling, some attempt has been made to attain uniformity but this has not been pressed Where so wide a circle of collaborators is involved, some variation in usage is unavoidable American spelling has been adhered to throughout, but beyond this the reader must accept some variability Since no general and completely accepted steroid nomenclature is in use (see Chapter XIII) the Editors have left the final decisions to the individual authors Certain variations in nomenclature will, therefore, be apparent, but these are in our opinion of minor importance

The Editors sincerely hope that this work will make a contribution to the orderly assemblage of knowledge that is essential to scholarship and understanding in a widely ramified branch of science

GREGORY PINCUS
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June 15 1948

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Chapter I

Historical Introduction

By GREGORY PINCUS and KENNETH V THIMANN

The concept of internal secretions or special tissue "ferments" in animals was advanced as early as 1775 by de Bordeu of Montpellier and extended by Le Gallois in 1801 The experimental demonstration of an internal secretion was first given by Berthold in 1849, in his classical experiment in which castration effects were prevented by the transplantation of testis tissue in the fowl Many other demonstrations followed Yet the understanding of the precise nature of a hormone, and also of the general principles involved, waited until the Bayliss and Starling work on control of pancreatic secretion by the intestine, in 1904 It was only then that the concept of a "chemical messenger" which coordinates the activity of different parts of the body was crystallized The reason for the delay in comprehension of the principle was concisely put by Hopkins "Up to near the end of the last century nearly every expert looked to the influence of the nervous system alone as concerned with the co-ordination of functions in the body, the conception of chemical regulation and co-ordination had achieved no place in the minds of the majority " In other words chemical physiology was a late comer to a field in which anatomy, morphology, and surgery had established themselves It is worth noting that, in such activities as color changes in the skin of fish due to melanophores, the relative importance of nervous and hormonal control is a matter of dispute even today

As with the concepts, so with the materials Eighty-six years after Berthold's work Laqueur isolated testosterone from bull testicles to bring forth the final chemical proof for the existence of the type of substance indicated by Berthold's experiments For other glands there are similar, but not quite so long, latent periods between the first effective experimental demonstration of internal secretory function and the isolation of a pure active hormone In Table I we list these occasions for those glands from which authentic chemical isolation has been made In the last instance listed, the isolation was made from quite other sources than were used for the original demonstration

During these "latent" periods there has, of course, been no lack of

TABLE I

THE LATENT PERIOD BETWEEN DISCOVERY AND HORMONE ISOLATION

Endocrine gland	First experimental demonstration of endocrine activity	*Isolation of a* pure hormone	Latent period, years
Testis	Berthold—1849	Laqueur—1935	86
Adrenal cortex	Brown-Séquard—1856	Mason, Myers, and Kendall—1936	80
Thyroid	Schiff—1856	Kendall—1914	58
Ovary (estrogen)	Knauer—1896	MacCorquodale, Thayer, Doisy—1935	39
Pancreas	Von Mehring and Minkowski—1890	Abel, Geiling, Rouiller, Bell, Wintersteiner—1926	36
Posterior pituitary	Oliver and Shafer—1895	Kamm, Aldrich, Grote, Rowe, Bugbie—1928	33
Adrenal medulla	Pellacini and Foa—1869	Takamine—1901	32
Corpus luteum of ovary	Ancel and Bouin—1910	Butenandt, Allen, Wintersteiner, Slotta, Ruschig, Fels, Hartman, Wettstein, Hisaw, Fevold—1934	24
Tip of oat seedling	Boysen-Jensen—1910	Kögl and Haagen Smit 1931	21

investigative activity Indeed they have frequently been marked by activity far more significant than the bounding investigations One has only to recall the effective discovery of pancreatic insulin by Banting, Best, and their co-workers, the penetrating investigations of estrus cycles by Marshall, Stockard, and Papanicolou, Long, Evans, and Allen, the laborious elucidation of testis function by Koch, Moore and others, and the quantitative explanation of plant tropisms by Cholodny, Dolk, and Went, as isolated instances of profound advancements in our knowledge of hormone physiology and biochemistry

Nor are we certain that the isolation of a pure active principle necessarily informs us of the true nature of the hormones at work in the organism Whether the posterior pituitary hormone is unitary or dual in nature *is still a matter of uncertainty and Abel in 1923 may have* isolated the single effective substance The functioning of acetylcholine and adrenalin as neurohormones is notoriously controversial Although testosterone has been isolated from bull testis, Ruzicka has failed to obtain it from swine testis and even now workers are searching for other testicular androgens. Furthermore, we are not certain of the true state of any of the steroid hormones *in vivo* Evidence for a corticosteroid ascorbate has recently been presented, and the problem of protein-steroid

conjugates has scarcely been touched In plants, the auxin-proteins present a very similar case As a historic parallel one may question the authenticity of thyroxin as the true thyroid hormone in view of the isolation of active thyroprotein

The modern concept of internal secretions in animals enunciated by Brown-Séquard in 1889, and clarified and baptized by Bayliss and Starling, required and has received voluminous proof It was unfortunate that for a time endocrinology was somewhat suspect, a none too reputable step-child of physiology This was in large measure due to the exploitation of the idea of rejuvenation by hormones, an idea that had its inception also with Brown-Séquard The use of testis extracts, of testis transplants and various "rejuvenation" operations in men were obnoxiously publicized in the late 19th and early 20th century But along with these questionable outgrowths of endocrinology were activities representing solid contributions to our knowledge of the subject The painstaking researches that led to the establishment of a genuine science cannot, of course, be described here, many of them are outlined in the special chapters of this book

Table I should not be taken as a guide to the history of advances in animal hormone discovery As a reading of our chapter titles indicates, important secretory glands and their products are omitted Many hormones, particularly among the lower animals, are far from characterization, let alone isolation What Table I does illustrate is that in most cases at least two generations are required to establish a hormone as a biochemical entity During this time the work follows a broadly repeatable pattern, confirmation and extension of the first observations, study of the reactions of other tissues, the gradual emergence of methods of assay, standardization of these methods, and the chemical isolation Then begins a new series of experiments with the pure material or a synthetic substitute

There has, in the last decade, been a notable quickening of general interest in hormones for two reasons The first is that the organic chemistry of the hormones especially of the steroids has been remarkably elucidated Following the isolation of estrone in 1929 the organic chemists have not only isolated ovarian, adrenal, and testis steroids but have synthesized a number of the principal active substances The second basis of quickening interest has been the application of modern methods of protein chemistry to the elucidation of the protein hormones The problem of posterior pituitary hormones has been reattacked and real progress in the purification of anterior pituitary hormones has been made The importance of these advances in hormone chemistry l es not merely in the chemical facts themselves The opportunity is offered to

physiologists biochemists, and clinicians to investigate in their fields the properties and usefulness of definable reproducible materials For this reason there is no need for the type of controversy on the efficacy of various extracts that clouded the early years of endocrine research The availability of large amounts of pure hormone has led to intensive studies of their effects in every branch of the animal kingdom

Brown-Séquard was insistent that every tissue of the body produced hormones Since his day experimenters with animal hormones have largely concentrated on specific glands There appears however to be a return toward his original interests The fact that the gut produces hormones has long been a challenge to the notion of a specific secretory gland But now it is being recognized that the field is much wider The neurohumors are obviously special tissue products Insect hormones do not necessarily come from organs of specific glandular function And in the plants we find growth hormones produced in a variety of tissues none of which is merely a gland It is tempting to look for a cycle of accomplishment in any science

The final stage in the study of a hormone and its activity, if indeed the word 'final' can ever be applied in physiology is the elucidation of the mechanism of its action But this involves very profound biochemical questions some of them we are not even ready to ask let alone to answer Many of them involve problems of growth—control of the limited specific growth of a particular tissue like the vaginal epithelium or the corpus luteum Some are interrelated with general metabolism All must entail enzyme systems probably systems of which as yet we know nothing Can a steroid function for instance as a coenzyme? How can the utilization of potassium or calcium be influenced by traces of substances? The future of hormone research will thus be in large part bound up with the advances in general biochemistry and physiology although hormones will doubtless prove to be invaluable tools for the attack of these fundamental questions As yet it must be said that this aspect of the work is scarcely begun Nevertheless the accumulation of knowledge now available means that much progress along these lines is now possible Perhaps therefore the present is the ideal time to take stock of the material and to put our knowledge together in carefully considered and well digested form ready for further advances

Chapter II

Plant Growth Hormones

By KENNETH V THIMANN

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I Historical Development and Definitions[1]

The concept of hormones in plants developed from the study of *tropisms or curvatures*. *Growing shoots* typically curve toward a source of light (positive phototropism), and away from the earth (negative geotropism), while roots curve toward the earth (positive geotropism), and in some cases away from light (negative phototropism). Curvatures may also occur away from or toward wounds (traumatotropism), electrodes (electrotropism), water (hydrotropism), etc. All these curvatures depend fundamentally on a difference in growth rate between the two sides of a growing organ—the convex side grows faster than the concave.

The careful studies of Charles and Francis Darwin on the geotropism and phototropism of seedlings (72) made it clear that the perception of light and gravity is centered in the tip of the growing organ, thus phototropism of the coleoptile of *Phalaris* (a grass) was prevented completely by covering the extreme tip with a black paper cap. Nevertheless, the Darwins observed that the curvature in such tropisms is not restricted to the tip but spreads downward to the basal regions. They concluded that some influence is "transmitted" from the tip to the basal regions. Thirty years later Boysen-Jensen (41,42) showed that this influence can cross a discontinuity. He cut off the tips of *Avena* (oat) coleoptiles and stuck them on again *in situ* with gelatin. On now illuminating the tips, curvature appeared first in the tip and then also in the base. Evidently the influence which is transmitted must be of a "material nature." This experiment was repeated with numerous variations, refinements, and controls by Paál (239). More important, however, was the following experiment (done with *Coix* coleoptiles): the tip was cut off and then replaced not symmetrically, but a little to one side. Without any illumination the plant now curved so that the side in contact with the tip

[1] For a fuller treatment see Went and Thimann (360) Chapter 2 and also Boysen-Jensen (46) Chapter 1

was convex This side, therefore, grew more than the other, and Paál deduced that in the tip "a substance (or a mixture) is formed and internally secreted" This substance diffuses into the lower regions and controls growth there In normal growth, this substance would be symmetrically distributed, but curvature would be due to asymmetric distribution, caused in some way by the light (or gravity)

This conclusion led to experiments on normal, not curved, growth Söding (287,288) showed that indeed the tip controls straight growth of the part below it Decapitation slows the growth greatly, though after some hours there is an acceleration due to "regeneration of the physiological tip" in the apical part of the remaining shoot This regeneration was subsequently shown (78,288) to be due to production of the growth-promoting substance by the most apical remaining tissue

Extracts of various tissues mixed with agar and applied to one side of decapitated coleoptiles (294) gave no evidence of containing a growth substance, though the technique of these experiments was a valuable advance Certain enzyme extracts applied in agar did produce curvatures, however (Seubert, 269)

Finally, Went (347,348) placed cut-off coleoptile tips on agar and applied this agar to decaptitated coleoptiles This caused curvature, the side in contact with the agar being convex Evidently the growth substance, although it could not be extracted by crushing the tissue, would "diffuse" from the intact tip into agar The curvature was shown to be proportional, within limits, to the amount of growth substance in the agar, *i e*, to the number of the tips placed on each block and the length of time they had been in contact This procedure has formed the basis for the assay method described below, by means of which three naturally occurring substances of similar growth promoting action have been isolated and many aspects of growth physiology have been studied The growth hormones have been named "auxins" and this name has since been applied to the whole group of synthetic substances of similar activity

The remaining historical development will be treated in the appropriate sections

Definitions

Considerable confusion in the use of the terms growth substance, growth hormone, growth regulator, Wuchsstoff, phytohormone, formative substance, and auxin has arisen The following definitions, which are practical rather than rigid, are put forward to simplify the situation [2]

[2] Similar, but not identical, definitions have been proposed by van Overbeek (234a)

Auxin *An organic substance which promotes growth (i e , irreversible increase in volume) along the longitudinal axis, when applied in low concentrations to shoots of plants freed as far as practical from their own inherent growth-promoting substance Auxins may, and generally do, have other properties but this one is critical* The definition excludes nutrient salts, and in order to exclude sugar, which unquestionably promotes longitudinal growth, the term "low concentrations" may conveniently be interpreted as "below *M*/1000" Most auxins produce clear-cut growth effects at 10^{-8} *M* or even considerably below [3]

Phytohormone *An organic substance produced naturally in higher plants, controlling growth or other physiological functions at a site remote from its place of production, and active in minute amounts* This definition includes those auxins which are of natural occurrence, certain of the vitamins, and other hormones such as those stimulating wound growth or the postulated hormones of flowering, etc

II Assay Methods

Like vitamin assays, auxin assays can only be reliably carried out with auxin-deficient test objects The most convenient of these is the dark-grown oat coleoptile from which the tip has been removed

A *Avena* Test

As developed by Went and modified by numerous workers this is carried out as follows

(*1*) Seeds of a pure line (the variety "Victory" or "Segerhavre" is the most commonly used) are husked, soaked for two hours in water, and laid out on wet filter paper with the embryo upward for 24 hours at 25°C in weak red light

(*2*) When the roots are about 2 mm long they are planted in glass holders (see Fig 1) with the root dipping into water contained in a zinc or glass trough The holders are held in brass clips in rows of twelve They can be adjusted in two planes so that the shoots can be made strictly vertical Some laboratories prefer to grow the plants in sand or soil, either in individual vials or in long narrow boxes

(*3*) The seedlings are allowed to grow for about 48 hours at 25° in a dark room The humidity must be controlled at 85–90% (relative) both to avoid drying and shrinkage of the agar blocks, and because plants grown in lower humidities are less sensitive (Gorter and Funke, 104), while at higher humidities guttation may occur Small cabinets have been designed to take the place of a controlled dark room (Avery *et al* 18) but the latter is more convenient

[3] Malic and other organic acids promote growth of the coleoptile at *M*/1000 and below (335a) but only in presence of auxin

(4) Straight seedlings of the same height are selected and the tips of the coleoptiles, to a length of about 1 mm , removed with sharp scissors (stage B in Fig 2) This and all subsequent operations are carried out

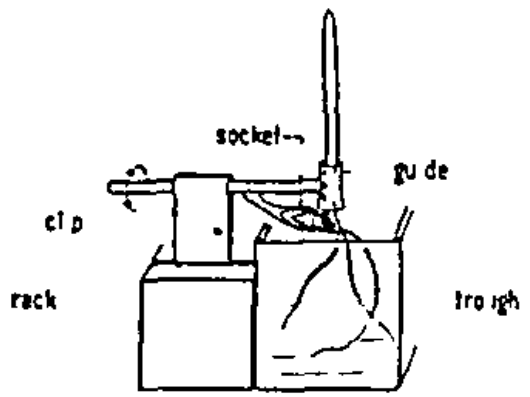


FIG 1 —*Avena* seedling in glass holder with roots in water Arrows show the directions in which adjustments can be made (From Went and Thimann 360)

in orange or red light free from wavelengths shorter than 5900 Å Shorter wavelengths, except at extremely low intensities produce phototropic curvature

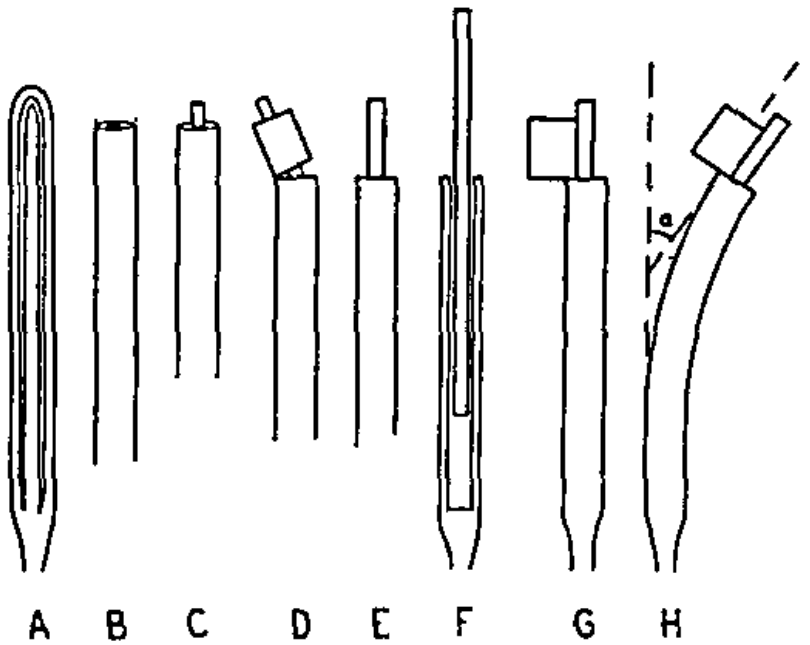


FIG 2 —Stages in the *Avena* test A The intact coleoptile with primary leaf within B First decapitation C Three hours later D E Second decapitation F Primary leaf pulled loose G Agar block in place H Curvature the angle measured is α (From Went and Thimann 360)

(5) Blocks of agar containing the substance to be tested are made by melting 3% agar and mixing with an equal volume of the test solution (Formerly blocks of pure washed agar were soaked in the solution but

this gives unreliable results) For experiments of the diffusion type, the plant parts are placed directly on 1 5% agar The blocks are cut up into small blocks of standard size, commonly 10 mm ³ The size is however, not critical since the curvature is essentially proportional rather to the concentration than the amount of the auxin contained in the block (Thimann and Bonner 318), with 10 mm ³ blocks 15% of the amount present enters the plant

(6) *Three hours after decapitation, when growth has slowed down* and regeneration of the physiological tip begun (see pp 12 32) a further 4 mm is cut off (stage D Fig 2) This is preferably done with special scissors with adjustable closure (see Went and Thimann 360, Fig 12) The protruding primary leaf is pulled until it breaks off at the base, but left inside the coleoptile as a support (stage F, Fig 2)

(7) The agar blocks are placed on one side of the decapitated coleoptile resting against the leaf (stage G Fig 2) From six to twelve or more plants are used for each determination After a standard time (90 *or 110 min*) *shadowgraphs of the resulting curvatures (stage H, Fig 2)* are taken This time is set by the "regeneration of the physiological tip' which causes formation of auxin on both sides and consequent regression of the curvature with increased growth rate

(8) The curvatures are measured in degrees with a simple goniometer, and from the averages the concentration of auxin in standard units is determined The plants for each test are calibrated by using blocks containing 0 025 mg indoleacetic acid per liter of agar, which gives a curvature within the range of proportionality, and a concentration five times higher which gives the maximum curvature obtainable The relation between concentration of auxin and curvature depends on the agar concentrations and the method of preparation of the plants For times age and conditions similar to those given above, this relation is shown in Fig 3 With higher agar concentrations the proportionality curve does not pass through the origin with lower concentrations the curve is convex to the abscissa (326) The curvature may also be expressed in terms of d the difference in growth between the two sides This is done by measuring r, the radius of curvature, and l, the length of the curved zone by means of a series of circular arcs cut out of paper Then

$$d = \frac{tl}{r}$$

where t is the thickness of the coleoptile, usually about 1 5 mm This method was introduced by Purdy (244) and is used mainly by Boysen Jensen and co workers who also grow their plants in sand or soil rather

than in glass holders The relationship between d (in mm) and curvature (in degrees) is approximately linear, a d value of 1 being about 38 5°

Although the dark-room conditions are essentially constant, the sensitivity of the test varies with the time of day, being highest in the early morning (167,360) In spite of several attempts (167), no explanation has been found for this In carrying out the test in diffuse light,

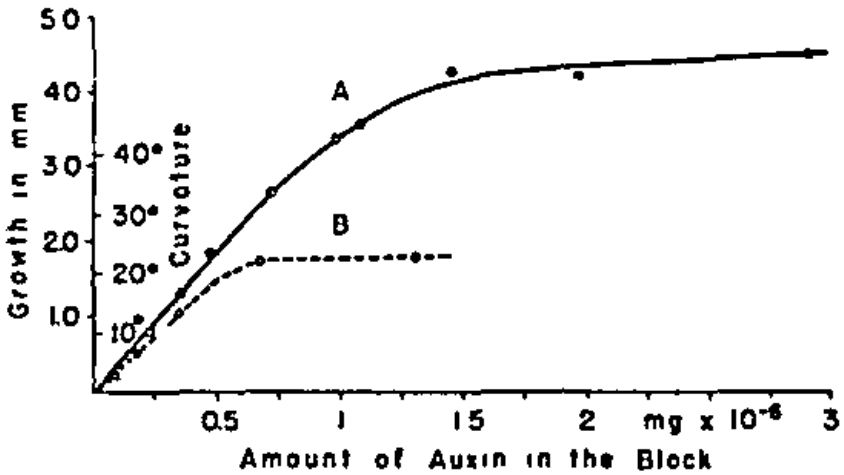


FIG 3 —Curvature (dotted) and straight growth (solid line) of *Avena* coleoptiles as a function of the amount of auxin applied (After Thimann and Bonner 319)

Söding and Funke (293b) found the sensitivity lower in warm weather than in cold, although this is not a direct effect of temperature

B Other Curvature Tests Using Agar Blocks

The characteristic feature of the *Avena* test is the use of an agar block of small volume This makes possible the determination of very small quantities of auxin In the standard test above, 0 025 mg indoleacetic acid per liter of agar gives a curvature of about 10°, measurable to about 10% The amount of indoleacetic acid in each block of volume 10 mm^3 is thus $2\,5 \times 10^{-7}$ mg or 0 0025 γ

The "deseeded" test (Skoog, 271) uses the oat coleoptile as above, but the seeds, *i e*, the endosperms, are removed, without damaging the embryo, at twelve to eighteen hours before the test The plants are held in the holders by cotton wool Since the endosperm of the seed furnishes the precursor which is converted to auxin in the regenerated physiological tip (see pp 22–24), these seedlings do not show regeneration Hence, the curvatures continue to increase up to six hours after application, and consequently the test, if curvatures are recorded at six hours, is three to five times as sensitive as the ordinary *Avena* test

The *Cephalaria test* (Söding, 291,293) is carried out in diffuse daylight with decapitated hypocotyls of *Cephalaria* Because this seedling has a

solid structure, unlike the hollow coleoptile of the grasses, it is less easy to apply the agar to one side Accordingly the hypocotyl is cut through obliquely and the block placed at the lower end of the cut The sensitivity of the test has an unaccountably large variation with the season *in June and July it is 400 times as sensitive as the Avena test, but in* winter it is only about half as sensitive, according to Soding (293) It has been little used

A curvature test with *Raphanus* hypocotyls was worked out by van Overbeek (226) The two cotyledons were removed, and in their place agar blocks were applied to the petioles—plain agar to one and the test block to the other The curvatures were photographed after two hours

C Straight Growth Measurements

Since the action of auxins in nature is to control straight growth, it is in principle desirable that assays should be checked by straight growth measurements, if not actually founded upon them

Straight growth of rapidly growing organs is readily measured over short periods with a travelling microscope In this way Soding (288) demonstrated "regeneration," *i e*, renewed auxin formation in the coleoptile stump some hours after decapitation At Utrecht an automatically recording growth measuring device, or "auxanometer," has been used in some critical studies (*e g*, that of Dolk, 78) Measurements of enlarged photographs taken at intervals during growth were used by Thimann and Bonner (319) and showed, *inter alia*, that straight growth, like curvature, increases with the applied auxin concentration up to a clearly-defined maximum (see Fig 3) Straight growth of decapitated coleoptiles has been used for comparing the activity of different auxins (260) Decapitated *Lupinus* seedlings almost stop growth when exposed to light, and if auxin is applied to them, the resultant elongation, for whole hypocotyls, is linearly proportional to the logarithm of the concentration (108), while short sections show a direct linear relationship very much like that of Fig 3 (Dijkman, 76)

Straight growth of isolated coleoptile sections is conveniently measured by placing the sections on fine glass rods (Bonner, 30, Thimann 310) or better still on the teeth of fine combs (Schneider, 262) and floating these on the test solution (see Fig 4) Sections of coleoptiles growing vertically on agar have been used by Monselise (209) and this method can be used with the auxin in agar blocks like the tests in B and C above Decapitated, isolated coleoptile sections, growing vertically, have also been used by Funke (88a) for assay of the growth inhibitor of maize seeds

When the sections are used in solution the pH must be brought to 6 0, because acid pH increases the growth by increasing the fraction of

the auxin in undissociated (as opposed to salt) form (31,259a) The sections should not be submerged in the solution but should break the surface (335a)

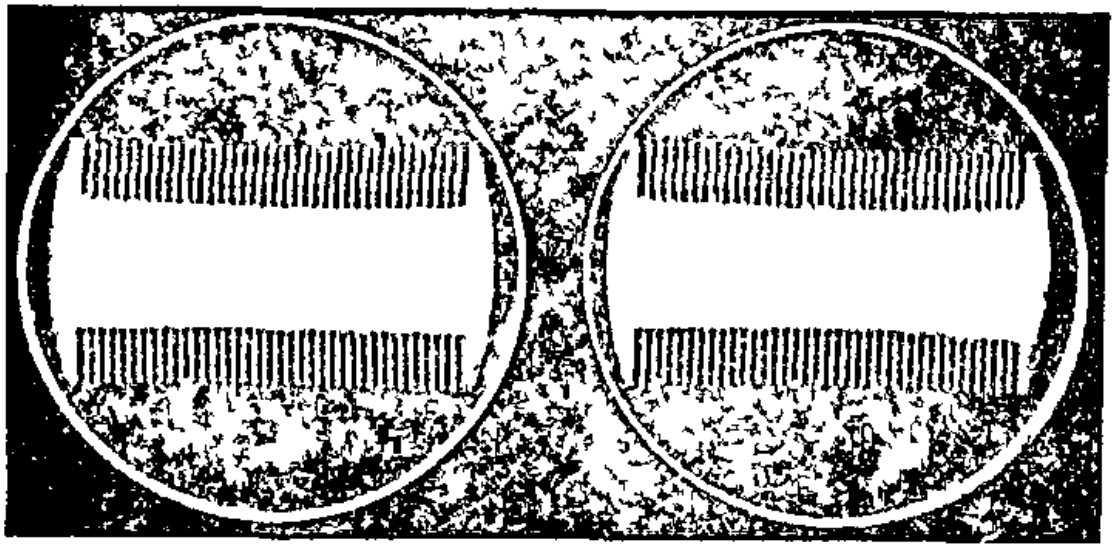


FIG 4 —Sections 3 mm long cut from coleoptiles mounted on combs and immersed in solutions in petri dishes photographed after 90 hours Left sections in water elongation about 10% Right Sections in auxin sucrose and KCl elongation about 100% with some growth in thickness (From Schneider 262)

D CURVATURE OF SLIT ORGANS

It was found by Went (351) that the internodes of pea stems, if slit lengthwise and immersed in auxin solutions, curve inward (toward one another), the curvature being more nearly proportional to the logarithm of the auxin concentration than to the concentration itself Jost and Reiss (150) used slit dandelion flowerstalks, and Thimann and Schneider (328) found slit coleoptiles of oats or corn very sensitive *Helianthus* hypocotyls have been used by several workers especially Diehl *et al* (75) but with the auxin applied in lanolin paste With all such objects, in water alone the halves curve outward, due to tissue tension In very dilute auxin solutions there is often a slightly increased outward curvature, more marked with some auxins than with others (327) Acid pH has the same effect, probably due to liberation of auxin at the cut surface (31) The inward curvature is, like the curvature of *Avena* coleoptiles in the test under A above, due to a difference in growth between the two sides of the organ, the outer side growing more than the inner (van Overbeek and Went, 238), but in this case the auxin is applied symmetrically and the differential response is inherent in the plant tissue Van Overbeek and Went concluded that the curvature is due to differ

ences in the rates of auxin entry on the two sides, entry taking place more readily through the outer intact side than through the central (wounded) tissue, but this has been disproved by Jost (149) and Thimann and Schneider (327), and the exact cause of the differential response has been the subject of considerable study It is clear that it involves (*a*) a true difference in the ability of the different layers of tissue to grow, the epidermis and outer cortex growing more, in response to auxin, than the pith and central layers (75,149 327), (*b*) a retarding effect on growth induced by the longitudinal wounding (263,353) The response of the epidermis is particularly important, "peeled" plants giving consistently smaller curvatures The different response to auxin of the different layers is the main cause of the curvatures, and is also responsible for the development of the tissue tensions in the normal growing stem The method is convenient where sufficient quantity of solution is available, and has been used in chemical studies on the activity of synthetic auxins (see Section III, C) It can be carried out in diffuse daylight

A modification in which coleoptiles are slit into quarters instead of halves (328) gives considerably greater sensitivity According to van Santen (259a) this method is much more sensitive to auxin *a* than to indoleacetic acid, but this is open to question

E Epinastic Curvature of Petioles

In many dicotyledonous plants, the angle between the stem and the petiole is constant and characteristic, provided the plant is vertical Application of auxin dissolved in lanolin to the upper side of the petiole will cause it to be depressed and the increased angle between stem and petiole can thus serve as basis for an assay method Hitchcock (136) and Hitchcock and Zimmerman (138) have used this method with tobacco and other plants (The curvature of the petiole in nature is classified as an epinasty and not a geotropism because, although caused by gravity it is not a curvature toward or away from the earth, but toward or away from the stem The direction of curvature is thus determined by the structure of the organs concerned) It is to be presumed that the curvature is due to acceleration of growth on the side to which auxin is applied as in the other tests above though analysis of the curvature in this sense has not been made It is well to point out that tests such as this with intact green plants growing in the light are open to an important objection, namely that the test object is already rich in auxin so that applied substances, even if they have no true activity, may give an effect through an action on the auxin already present It is probably for this reason that some organic acids which are not auxins at all, show activity in this method Relative activities

of different auxins in causing epinasty are roughly in the same order as for causing curvature and gall formation in green plants, but not the same as for the *Avena* test (122)

F Other Methods

Methods depending on the formation or inhibition of roots or buds will be discussed in appropriate sections below A few of these have been used as assay methods in the past but at present they are used mainly in the studies of the phenomena concerned and not as assays *Avena* coleoptiles have occasionally been used, intact or decapitated, with the auxin applied in lanolin, the sensitivity is 10–50 times less than with agar (Brecht, 50, Avery *et al*, 14) In many cases it is desired to assay for a particular type of activity such as growth inhibition of shoots (Section VII, D) or parthenocarpic fruit formation (Section VIII, B) Certain auxins, particularly the alkyl esters of the acids, are effective in the vapor form (375), their action has been assayed by epinasty (above) or by morphogenetic effect on developing buds (see Section III of Chapter III) The swellings produced by applying auxin in lanolin to the decapitated stems of *Vicia faba* seedlings have been utilized for an assay method by Laibach and Fischnich (182) The increase in diameter, measured after four days in the dark, is proportional to the logarithm of the auxin concentration up to a limiting value

III. Chemistry of Auxins

A "Auxin *A* and *B*"

In view of the importance of the coleoptiles of the grasses, especially oats, in the early work, it would be expected that efforts would be made to isolate auxins from this material However, the quantities present are far too small First steps toward isolation were made by the discovery of auxin in various commercial enzyme preparations by Seubert (269), in cultures of several fungi including *Rhizopus suinus* by Nielsen (216,217), and in human urine by Kogl and Haagen Smit (162 *cf* 164)

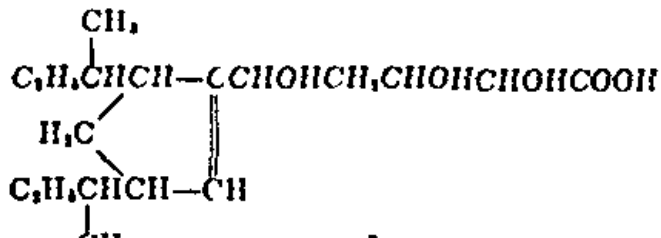
From the ether-soluble fraction of acidified urine, by an extensive series of fractionations, involving a concentration of 21,000 times, Kögl, Haagen Smit, and Erxleben (163) isolated an acid, termed "auxin *a*," $C_{18}H_{32}O_5$, and also its lactone Turning their attention to plant material they analyzed a number of samples of cereal seeds and selected a corn germ oil and a malt sample which appeared to have very high auxin contents From these were isolated both the auxin *a* above and a new acid, $C_{18}H_{30}O_4$, named "auxin *b*" (160) The degree of concentration required was 100,000 times for the malt and 300,000 times for the corn

oil The two substances are closely related, the former being a trihydroxy and the latter a ketohydroxy acid:

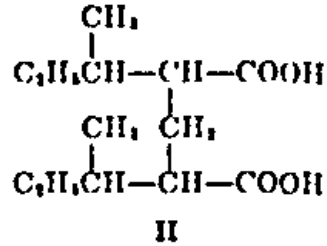
$(C_{11}H_{21})CHOHCH_2(CHOH)_2COOH$	Auxentriolic acid, "auxin *a*"
$(C_{11}H_{21})CHOHCH_2COCH_2COOH$	Auxenolonic acid, "auxin *b*"

The "auxin *a*" lactone is considered to have the 1,5-lactone form

In spite of the small amounts available (less than 1 g of total active crystals), Kögl and Erxleben established the structure of the C_{11} residue as 2,4-di-*sec*-butyl-Δ^1-cyclopentene and confirmed this by the identity of the substituted glutaric acid, obtained by oxidative breakdown, with a synthetic product, 2,3-diisobutylglutaric acid, II The full formula of auxin *a* is therefore I



I



II

On standing in the dark, the double bond shifts to the side chain and the hydroxyl to the ring to produce an inactive substance, pseudoauxin *a* (p 19) Auxin *a* lactone undergoes a similar change with loss of water This and related changes may play a role in phototropism (see Section V)

B Indole-3-Acetic Acid

In extending their work on urine, Kögl, Haagen Smit, and Erxleben (165) found that a large part of the auxin present was destroyed by attempts to lactonize with hydrochloric acid in methanol A modified isolation method thereupon led to the identification of indole-3-acetic acid as a third active compound Its activity in the *Avena* test is probably about half that of auxin *a* or *b* Kogl and Kostermans (168) also isolated this substance from yeast plasmolyzate

Working independently on the auxin produced by *Rhizopus suinus* cultures, Thimann (310) showed by isolation that this also is indole-3-acetic acid At first it was thought that indoleacetic acid is typically a

product of microorganisms and not a true hormone of higher plants, and it was accordingly named by Kögl *et al* "heteroauxin," but Haagen Smit and co-workers (125) subsequently isolated it in pure form from alkali hydrolyzed corn meal and indicated that most of the activity of the hydrolyzed meal was due to indoleacetic acid rather than auxin *a* Haagen Smit *et al*, (124) later obtained it also from the endosperm of immature corn grains Gordon and Wildman (102 103) have brought forward evidence that alkali treatment produces traces of indoleacetic acid from the tryptophane in a number of proteins (see below) but this is not likely to be the main source of the indoleacetic acid isolated Instability to hot acid and stability to alkali indicate that the auxin extracted from many higher plants (237, and see Section D below) is of the indole type It is probable therefore that indoleacetic is widely distributed in higher plants perhaps more widely than auxin *a* and *b* and it is evidently a true plant hormone The high specificity of the indoleacetic acid inactivating enzyme of the pea plant (306) also points in this direction some workers believe auxin *a* and *b* occur only rarely

Besides indoleacetic acid, indoleacetaldehyde also occurs in plants particularly in dark grown *Pisum Vicia Helianthus* and *Brassica* (187) The aldehyde is readily oxidized to the acid by Schardinger s enzyme from milk, or by contact with soil It behaves as a "neutral auxin," and was discovered through its presence in the neutral fraction by Larsen who purified extracts by shaking out from ether at different pH Its identity was established by conversion to indoleacetic acid and various other tests Its widespread occurrence is of course, further evidence for the importance of indole derivatives as plant hormones

C Synthetic Substances Not Known to Occur Naturally

A great number of related compounds have been prepared and tested The results depend to some extent on the assay used The *Avena* test is highly specific Besides the above compounds only the lower alkyl esters and two of the methyl derivatives of indoleacetic acid (169), the isostere indene-3 acetic acid (311) and indole-3-butyric and 1 naphthaleneacetic acids (360), show appreciable activity in this test The potassium or sodium salts show about the same activity as the free acids provided the solutions are not buffered A few other substances show activity in very high concentrations only, frequently producing very short apical curved zones Phenylbutyric acid, which is inactive by itself, inhibits the effect of indoleacetic acid, by competition (272a) or perhaps by a more complex mechanism This substance (and also cyclohexaneacetic acid) greatly increases the auxin curvature in the pea test (353) This is explained by Went (353 356) in terms of two proces

ses, one, the "preparatory" reaction, can be carried out by substances inactive as auxins, while the other, the "growth" reaction proper, *requires the chemical structures discussed below*

Straight growth of isolated stem or coleoptile sections (see Section II, C) is less specific, and the curvature of immersed slit stems (Section II, D) or other methods still less so Hence the activity of a given synthetic auxin, relative to a standard such as indoleacetic acid, varies with the type of test This is illustrated by Table I (from Thimann and Schneider, 1939), which not only shows the difference in specificity of the tests, but also illustrates how compounds inactive, or almost so, in one test may show high activity in others However, the order in which the substances fall is nearly the same in each test (see also the data of Gustafson 122)

Using the slit pea stem curvatures, Haagen Smit and Went (126) and Koepfli, Thimann, and Went (157) have tabulated the activities of a large number of related compounds, and Veldstra (1944a) has added a number more Using epinasty and the changes in shape of young tomato leaves, Zimmerman and Hitchcock (372) and Zimmerman (370) have added a further large group, including the highly active ring

TABLE I

RELATIVE ACTIVITY OF SIX AUXINS*

Acid	Curvature of slit stems of *Pisum*	Straight growth of *Pisum* sections	Straight growth of *Avena* sections	Curvature in standard *Avena* test
Naphthalene-1 acetic	370	23	15	2 5
Indole-3-butyric	190	22	9	8
Indole-3-propionic	150	8	1 6	0 1
Phenylacetic	10	0 4	0 3	0 02
Benzofurane-3-acetic	6	0 3	0 11	0 02
Phenylbutyric	3	*0 08*	*0 06*	*0 005*

* Activity of indole-3 acetic acid brought to 100% for each test (*From Thimann* and Schneider, 328)

substituted derivatives *p*-chloro- and 2,4-dichlorophenoxyacetic acids Some approximate relative activities for the induction of seedless fruit (see Section VIII) have been given for these compounds by Zimmerman and Hitchcock (373) *As might be expected, the ratios of the activities* of various substances determined in this way are not the same as by the above methods

In spite of all this work, it is still not possible to make a *really binding*

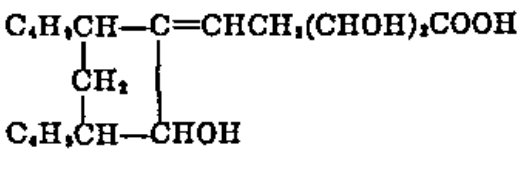
statement as to the structural requirements for auxin activity The difference between the tests, mentioned above, is in part due to the necessity for the substance to be transported through the plant tissue in tests using agar blocks, but not in tests using immersion in a solution Some substances, though highly active locally, are not readily transportable This important limitation, brought to light with indeneacetic and benzofuraneacetic acids by Thimann (311), was confirmed for several substances by Went and White (361) in transport experiments, which are discussed in Section IV, A Then, too, the stability of the substance to plant enzymes, its permeability through cell membranes, and the fraction present in undissociated form (29,31) all influence the responses, the last because the ionized salt form does not penetrate into the cell readily, as shown by Albaum *et al* (4) A correction for the extent of dissociation increases the apparent activity of many substances in the pea test The influences of these modifying factors are discussed in Went and Thimann, Chapter 8 (360), and by Went (353,355), and more recently by Veldstra (339) The auxin-inactivating enzyme in pea plants is highly specific for indoleacetic acid (Tang and Bonner, 306), this might cause this substance to show a lower activity than other, unnatural, compounds

These factors can as a first approximation all be considered secondary, the primary one being the ability to cause cell enlargement when present in the cell Using this criterion of primary activity, Koepfli, Thimann, and Went (157) stated the following structural requirements (*1*) A ring system as nucleus, (*2*) A double bond in the ring, (*3*) A side chain containing a carboxyl group (or an ester or amide readily convertible to a carboxyl), (*4*) A distance of at least one carbon atom between this group and the ring, and (*5*) A particular space relationship between the carboxyl and the ring

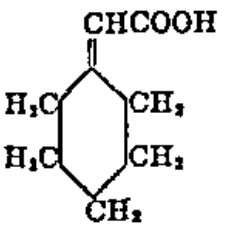
As to *1*, no aliphatic compounds tested have shown activity

As to *2*, dihydroindoleacetic acid and dihydroauxin *a* are inactive, so is cyclohexane acetic acid A number of compounds with unsaturation in the side chain but not in the ring, such as pseudoauxin *a*, III, cyclohexylideneacetic acid, IV, and benzofulveneacetic acid, V, are inactive

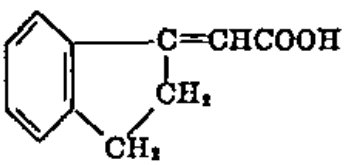
As to (3), some modification is needed to allow for the small but definite activity of naphthyl-1-nitromethane (*aci* form), VII, and indican,



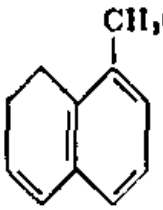
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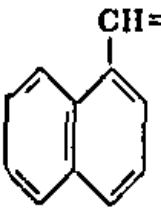
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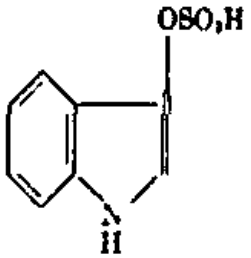
V



VI
Naphthalene 1 acetic acid

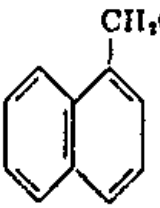


VII
Naphthalene 1 nitro (aci) methane



VIII
Indican

VIII, both of which have acid side chains which are not carboxyl groups (339) It may be that any acidic (i e , hydrogen-ion-yielding) group is effective to some extent Also naphthaleneacetonitrile and tryptamine (271) show a slow activity, which is doubtless due to conversion to a carboxylic group within the plant There is some evidence, however, that naphthalene-1-acetamide IX, *is active without being hydrolyzed (335a)*

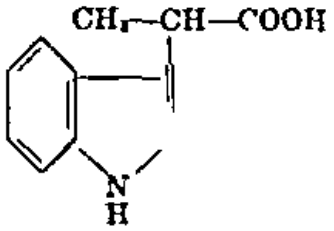


IX

The activity of esters is not entirely clear The data of Kögl and Kostermans (169), with the *Avena* test, show decreasing activity with increasing molecular weight of the alkyl-esterifying group of indoleacetic acid, they therefore concluded that activity was due to hydrolysis (by plant esterases) to the free acid, which should go with decreasing rapidity as molecular weight of the alkyl group increases Avery *et al* (14) have found the esters to have about the same activity as the free acids, or somewhat less in the case of naphthaleneacetic acid, this would agree with the above view However, Zimmerman and Hitchcock (371,374) found, in experiments with tomato plants, that at least the methyl ester of indolebutyric acid has slightly higher activity than the free acid This might of course be due to some secondary property of the ester *such as* ease of penetration through the intact epidermis By contrast, the esters of auxin *a* are inactive in the *Avena* test (160)

As to *4*, the optimum distance is commonly one carbon atom, activity decreasing with increasing length of side chain, but there is some alternating effect, indolebutyric being more active than indolepropionic acid *The carbon atom may be replaced by other hetero atoms In the case* of phenoxy and napthoxy acids the hetero atom oxygen is present as well as the one carbon atom

Point *5* is the most ill defined. The activity of *cis*-cinnamic acid and some of its derivatives, and the inactivity of the *trans* isomers, are among the main pieces of evidence. In the *Avena* test, the two optical isomers of α-(β-indole)-propionic acid, X, have different activity, the (+) being thirty times as active as the (−) (171), but, since the activity on *immersed*



X

coleoptile sections is identical, this difference apparently does not relate to *primary* growth activity. It provides another example of the high specificity of the *Avena* test Veldstra (339) has postulated that the side chain must be perpendicular to the plane of the ring, and supports the argument by consideration of molecular models. He makes clear that in *cis*-cinnamic acid the side chain is perpendicular to the plane of the ring, while in the *trans* form both are in the same plane Even in napthalene-1-acetic acid the position perpendicular to the ring is favored Yet it is difficult to see how introduction of halogen atoms into the ring could alter such spatial relationships Thus Zimmerman showed, with epinasty (370), that introduction of the ortho chlorine atom increased the activity of phenoxyacetic acid twenty times, the para chlorine atom by eighty times, while both together (2,4-dichloro derivative) increase it some 1200 times. In the pea test (335a) these four substances have the following activities, as per cent of that of indoleacetic acid

Phenoxyacetic acid	*ca* 0
o-Chlorophenoxyacetic acid	4
p-Chlorophenoxyacetic acid	200
2,4-Dichlorophenoxyacetic acid	1200

While substitution in the ortho position might possibly have some effect *in orientation of the side chain, it seems hardly likely that substitution* in the para position could do so. There are numerous other examples of the same effect. The exact nature of the spatial relationships must therefore be left open for the present.

D. Nature of Auxin Precursors

The auxin in human urine clearly comes from the diet. The esters of auxin *a* are inactive, and some oils yield auxin on hydrolysis with

lipase or with sodium ethylate (160) Ingestion of natural oils increased the auxin content of urine, while hydrogenated oils, pure protein, and sugar did not (163) Indoleacetic acid in urine, similarly, comes from ingested protein (125), wheat giving a particularly clear rise in urine auxin as soon as one hour after feeding

As was mentioned in Section II, the auxin produced in the coleoptile tip, or in the apical part of the stump in "regeneration," is formed from a precursor in the seed This was first made probable by Cholodny (61), who showed that soon after the seed was wetted auxin appeared This auxin does not, as claimed by Pohl (242), travel directly up into the coleoptile, but that which goes into the coleoptile tip travels up as an inactive precursor This was shown by Skoog (271), who placed agar blocks for a while on the stump of decapitated coleoptiles and then showed that when applied one sidedly to freshly decapitated coleoptiles ("deseeded test") they caused no effect at first but slowly induced a curvature after two to six hours (341)

Following the work of Thimann and Skoog (332), Gustafson (115,118), Wildman and Gordon (365), and Thimann Skoog and Byer (333) on the extraction of auxin from plant tissues, it has now become increasingly clear that many plant materials yield auxin very slowly on extraction with ether, and that this auxin stems from proteins in the tissue The slow yield is due to a reaction with water, probably proteolysis, which liberates auxin This reaction is stopped by boiling (332) and this has been put to use for an assay of the free auxin in plant tissues by Gustafson (118) It is also stopped by thorough drying (197,332) and at once resumed on adding water Proteolytic enzymes, especially chymotrypsin, were found by Skoog and Thimann (274) to accelerate greatly the liberation of the auxin Wildman and Gordon (365) and Gordon and Wildman (103) have obtained an auxin which is almost certainly indoleacetic acid from isolated plant proteins both from leaves and from seeds Since this auxin is best obtained by alkaline hydrolysis (25), some of it at least, doubtless derives from oxidative deamination of tryptophan However, this is probably not the whole story, for two good reasons (*a*) in the case of cabbage leaves the auxin yields are probably too high to be ascribed to the tryptophan present, according to the determinations of Avery, Berger, and White (15), and (*b*) auxins which may be either acid labile (*i e*, indole derivatives) or alkali labile (presumably auxin *a* or *b*) may be obtained from purified wheat proteins (Gordon 102) It is to be noted that Gordon's wheat proteins were well characterized, which makes it highly improbable that the auxin could be merely an impurity' In any event particularly in the case of auxins liberated by enzymes there is no reason to doubt that, as was originally

postulated (333), true auxin-protein complexes do occur These could, of course, serve as important auxin reserves for the plant

The form in which auxin occurs in seeds differs from that in other tissues The bulk of the auxin in the cereal grains is in bound form, in the endosperm, and only liberated by alkaline hydrolysis (13,125,130, 333) It is this material which is indoleacetic acid, as shown by Haagen Smit *et al* (124,125) and Berger and Avery (24,25) The quantities are large enough in corn, *i e* 20–100 mg /kg , that it acts as an antivitamin in animal growth (156) However, some auxin is obtainable, largely from the embryo, by direct extraction with organic solvents, as in the isolations by Kögl and co-workers described above and this material is auxin *a* and *b* Thirdly, the addition of water to the endosperm liberates a moderate quantity, presumably by enzymic action Much of this was probably also bound in the dry state, either chemically as a precursor, or in some physical or adsorptive manner, as in dried *Lemna*, in which the auxin can be first liberated and then made unextractable by drying (332) Whether the water-extractable auxin in the grain is auxin *a* or indoleacetic acid is not clear Hatcher (130) has assumed that it represents *free auxin, the alkali hydrolyzable part being the bound or precursor* form, but there is not enough evidence for this yet The situation is complicated by Funke's finding (88a) that part of the auxin in corn endosperm is stable to hydrogen peroxide.

In contrast to the grains no auxin is liberated from *Lemna* by alkali autoclaving although as with other green tissues, it is set free slowly by moist ether (118,332) as discussed above Cabbage (15) and spinach, however, do yield some auxin to alkali, though in the author's unpublished experiments spinach leaf proteins gave much higher yields with chymotrypsin than with alkali The purified auxin protein in spinach leaf cytoplasm does not liberate its auxin readily, it is resistant even to vigorous electrodialysis and sets free auxin only when actual proteolysis occurs, so that it is indeed a relatively stable complex (Bonner and Wildman, 35) Between these two extremes there seem to be many intermediate states, in different tissues, in regard to ease of liberation (309,333)

A true precursor, of course, would be a substance from which auxin is continually produced, by plant enzymes, under normal conditions and in physiologically significant amounts It is by no means certain that the auxin proteins fulfil these criteria Neither papain-hydrogen cyanide nor the enzymes of autolyzing *Lemna* liberated any appreciable amount of auxin (333), and chymotrypsin, as far as is known, does not occur in plants Ficin, which does liberate auxin from *Lemna*, is a plant enzyme, it is true, but it is not known to be widely distributed The partial

liberation of auxin on slow drying of leaves may be enzymic, but it is quantitatively rather small A true precursor system was, however, studied by van Overbeek (233) in the isolated coleoptile tip, which continues for a long period to yield auxin to agar blocks, although the amount which can be extracted from it by organic solvents at any time is relatively small (309) Berger and Avery (23,24) made a partial isolation of a true auxin precursor from corn; this appears not to be a protein, having only 4 7 to 6 4% nitrogen, but its nature remains unknown It yields indoleacetic acid on alkaline hydrolysis. The variation in amount of precursor and "free auxin" (but see comment above) with age and drying of the grain in rye has been very thoroughly studied by Hatcher (130), who finds that the "free" form appears first and then decreases as the bound form increases and the grain ripens Some of these changes may, however, be due to variations in the amounts of inhibiting substances rather than in the true auxin (88a).

A more remarkable precursor was obtained earlier from radishes by Stewart, Bergren, and Redemann (297,299); this substance in the intact form actually inhibits growth of the *Avena* coleoptile, giving marked *positive* curvatures, but on hydrolysis yields an auxin which is probably indoleacetic acid Its chemical nature is also unknown but it is thought to be a peptide The further study of this substance might be important in regard to inhibitions (see Section VII).

The ability to convert tryptophan to indoleacetic acid is probably widespread among microorganisms; this is doubtless the source of the auxin in fungus cultures, as shown by Thimann (310). Furthermore, this is the most probable source of the large amounts of auxin produced in bacterial infections of plants such as legume root nodules and crown galls (see Section VIII, A) Other plant infections resulting in pathological overgrowth (188,189) may have the same explanation, and indeed Link *et al* (197) have shown that aphids are very rich in auxin; whether this was extracted from the leaves on which they fed, or elaborated within the aphid is not clear, but in any event either the removal or injection of auxin by the aphid may account for some of the growth effects caused by these parasites As to higher plants, the evidence as to their ability to convert tryptophan to indoleacetic acid under natural conditions is not perfect Tryptophan causes a slow curvature in the "deseeded" *Avena* test, it causes straight growth of coleoptiles when applied to the base but not to the tip and it leads to root formation on pea cuttings (335,335a) On the other hand it cannot replace indoleacetic acid in sterile tissue cultures, as found by Nobécourt (221) Unless the tests with higher plants are carried out under sterile conditions a positive result might always be due to infection Because the growth effects produced by tryptophan

differ anatomically from those caused by indoleacetic acid, Kraus (174) claims that its action cannot be due to conversion to the latter compound But since only one concentration, in lanolin, was studied in his experiments, and since growth effects are characteristically dependent on concentration, this conclusion is obviously unjustified The best evidence of conversion is that of Wildman *et al* (364a), who obtained formation of an active auxin by spinach leaves infiltrated with tryptophan within two to four hours The enzyme system was present in dialyzed cytoplasm prepared from the leaves and had its optimum pH at 7 5 There is some evidence that the reaction goes via indolepyruvic acid (*cf* 310), in any event it is an oxidative process

The case of tryptamine, which, like the precursor in the seed, is directly converted to auxin in the *Avena* coleoptile (271) is worth special mention, though its biological significance is not known Lastly the indoleacetaldehyde found by Larsen (187) in etiolated *Pisum* and other plants must be considered under this head Larsen s extracts of neutral auxin, which had quite low activity, were converted to highly active material, considered from diffusion measurements to be indoleacetic acid, by treatment with soil or with a preparation of Schardinger oxidase In some cases the neutral material had no growth activity at all, which suggests that there is more than one neutral compound convertible to indoleacetic acid There is no evidence here, though, that enzymes in the plant can carry out the conversion Hemberg (131a) finds a similar situation in potato tubers

An interesting general scheme for auxin activity has been proposed by Skoog, Schneider, and Malan (272a), according to which the auxin molecule, envisaged as a kind of coenzyme, has to combine on the one hand with its substrate and on the other with an apoenzyme Precursors could thus be of two kinds those in which the substrate-combining part is covered or distorted but can be corrected by the plant, *e g* , tryptamine or indoleacetaldehyde, and those in which the apoenzyme-combining part has been combined with some other molecule but can be freed under some conditions The latter have their substrate-combining activity intact and can therefore occupy the substrate to the exclusion of free auxin molecules, thus giving competitive inhibition (*e.g* , phenylbutyric acid) or even total inhibition (*e g* the inhibitor of Stewart *et al* described on p 24) These authors point out that, if excess auxin were present, some molecules would combine only with the substrate and some only with the apoenzyme "thus effectively blocking each other from reacting " This would account for inhibitions of the type discussed in Section VII, A This ingenious scheme has much to recommend it, though considerably more evidence would be needed to establish its validity

IV. Transport of Auxin

A Polar Transport and Its Mechanism

One of the most remarkable properties of living plant tissue is the strictly polar way in which auxin is transported in it The polarity of shoots, particularly in regard to bud development and root formation, has been recognized from very early times, and the polar transport of auxin provides an explanation for at least many such phenomena The earlier work on polar transport of auxin has been so fully reviewed (360, Chap 6) that it needs only the briefest recapitulation here

In seedlings phototropism is detected by the tip and the stimulus conducted toward the base, movement in the reverse direction does not

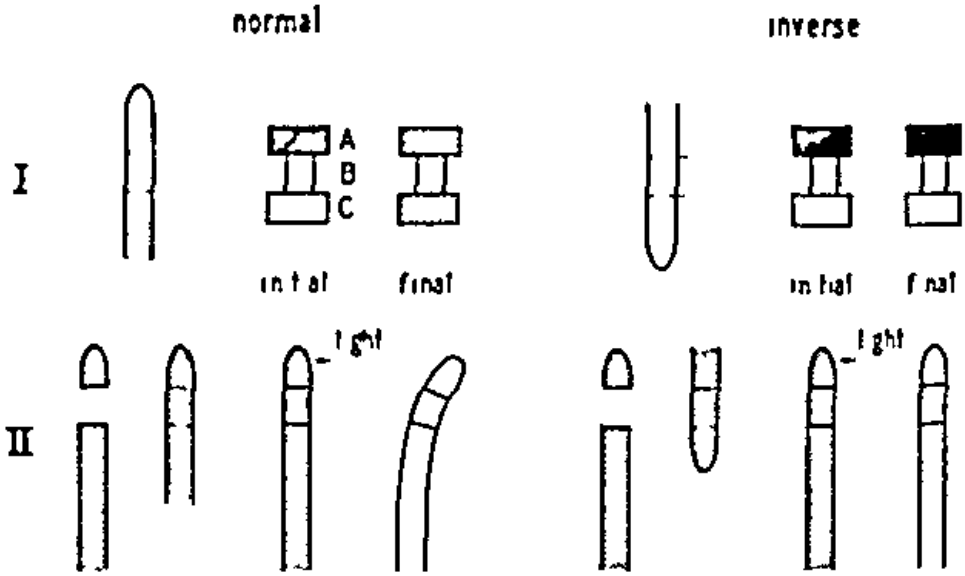


Fig 5 —I Diagram of transport experiment Auxin is transported from agar block A through coleoptile section B to receiving block C Left normal transport right section inverted no transport Degree of shading indicates auxin concentration in agar

II Transmission of phototropic stimulus through normal (left) and inverted (right) section of coleoptile introduced between tip and base of another coleoptile (From Went and Thimann 360)

occur Interposition between the tip and base of a section of inverted tissue prevents the movement (see Fig 5, II), which is therefore strictly acropetal Auxin will be transported directly through a short section of *Avena coleoptile in the apex to-base direction, but not inversely* (Fig 5, I) The process is not one of diffusion, as was proved by the experiments of van der Weij (346), which were carried out as shown in Fig 5, I, the auxin in the blocks being determined by the *Avena* test The main results can be summarized as follows

(*1*) The temperature coefficient of the amount transported per unit time between 0° and 30°C is about 3, *i e*, that of a chemical reaction (*2*) The velocity, however, as measured by the time taken for the first auxin to appear at the basal end of the conducting tissue, is about 12 mm /hour in *Avena* and is independent of temperature This is determined by extrapolation (see Fig 6) (*3*) The concentration of auxin in the agar block at the receiving end soon equals that in the donating block, and subsequently exceeds it, so that auxin must be *actively* transferred against its gradient (*4*) By etherizing the sections, polarity

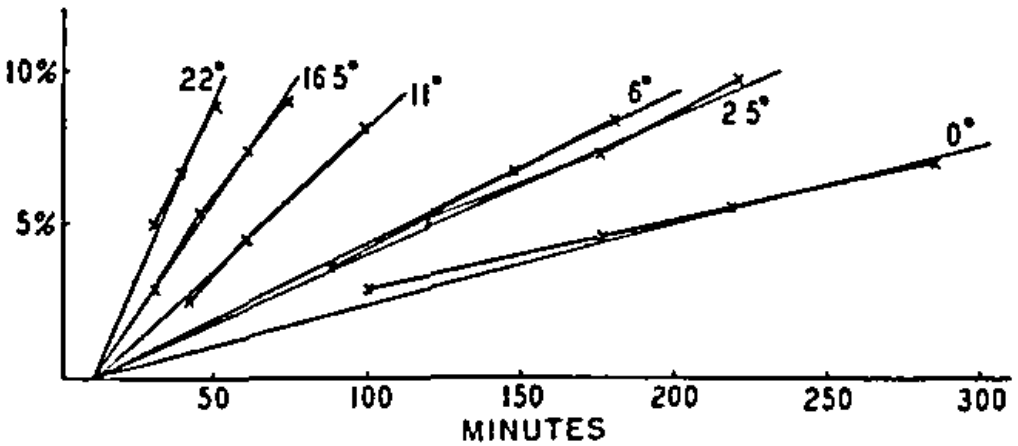


Fig 6 — Auxin transported as per cent of the amounts applied through 2-mm sections of *Avena* coleoptiles as a function of time in minutes Extrapolation of the curves of different temperatures indicates that they all cross the *x* axis at about 10 minutes showing that transport velocity is independent of temperature between 0° and 22°C (From van der Weij 346)

disappears and with it also disappears the "active" nature of the transport, it now becomes essentially a diffusion process

Auxin transport is thus like that of "objects along a moving band, the band goes at constant speed so that the number of objects arriving at the end per unit time is independent of the length, the time required for the first object to reach the end is proportional to the length of the band, if not removed from the end the objects continue to pile up" (Went and Thimann, 360) Stems (see Beal, 21), petioles, hypocotyls and leaf veins behave like coleoptiles so far as they have been studied [4] Tissue cultures, especially of carrot and endive, demonstrate the polarity of auxin transport in many ways (Gautheret, 98, pp 161–166) Other auxins than indoleacetic acid move both more slowly and in smaller quantities per unit time The data of Went and White (361) yield the following rates in millimeters per hour through *Avena* coleoptiles

[4] Unpublished experiments of W P Jacobs show that the polarity is far from strict in young *Phaseolus* hypocotyls

Indoleacetic acid	9 0
Indolebutyric acid	6 6
Anthraceneacetic acid	5 4
Napthaleneacetic acid	3 9
cis Cinnamic acid	Not detectable

It should be added that longitudinal transport of auxin is not affected by light (226), this is important for the understanding of phototropism (see Section V)

The mechanism by which this active transport is achieved is not understood Accumulation of solutes against a gradient, as by roots or by algae growing in very dilute nutrient solutions, must involve a comparable type of active transport (352), but in this case in the lateral rather than the longitudinal direction Arisz has recently brought to light (10) a similar transport of amino acids through the tentacles of *Drosera*, and Schumacher (265) described polar movement of fluorescein in stem hairs of cucurbits The polarity of auxin transport is therefore not an entirely isolated phenomenon

Attempts have been made to relate the transport to the electrical polarity of the plant The apex of shoots is in general negative to the base, as shown by the early work of Lund (see 200) with nonpolarizable electrodes This apical negativity is still present in short sections of stems or coleoptiles, and is largely abolished by etherization (64) The anion of a weak acid such as auxin would, of course, be transported from apex to base under such a potential Koch (153) showed that plant auxin in agar does in fact move toward the anode, and Clark (63) confirmed this for pure indoleacetic acid Kögl *et al* (167) showed essentially the same thing by making the agar block in the *Avena* test negative to the plant and passing a small current, which had the effect of increasing the resulting curvature, doubtless by increasing the movement of auxin from the agar into the plant Then, too, coleoptiles and shoots placed in air or water between oppositely charged poles curve toward the positive pole (6 49 153) such curvature implies more growth on the side toward the negative pole Electrolytic movement of auxin has even been produced directly in plant tissue by Koch (153), by inserting electrodes into opposite sides of sunflower hypocotyls, which were subsequently halved and tested for auxin (by applying them to roots) The hypocotyls here curved toward the negative pole and the convex half gave *the greater curvature on the test roots* These experiments all show that electrolytic movement is possible, and takes place in the right direction But here the parallel ends, for the following reasons (*1*) a potential gradient of 50 volts/cm was needed for detectable transport—far higher than the electrical gradients observed in plants, (*2*) externally

applied potentials do not affect the polarity of auxin transport through coleoptile sections, even though they may reverse the electric polarity, (3) inverting the section with respect to gravity inverts the electrical gradient but does not affect the auxin transport (63), and (4) treatment with 10–100 p p m of sodium glycocholate completely abolishes the transport but does not affect the electrical polarity, or indeed any other observable property of the coleoptile section (see Table II, from Clark, 64)

The absence of any effect of low glycocholate concentrations on respiration, while auxin transport is wholly prevented is of interest since

TABLE II

EFFECT OF GLYCOCHOLATE ON AUXIN TRANSPORT AND ELECTRICAL POLARITY OF COLEOPTILE SECTIONS

Sections infiltrated with	Units auxin transported in 2 hr	Emf between apex (−) and base (+) mv	Protoplasmic streaming	Q_{O_2} (in separate expts.)	Appearance of tissue
Water	11.4	10	+	1.21	Turgid
Na glycocholate					
10 p p m	0	10	+	1.22	Turgid
100 p p m	0	10	+		Turgid
1000 p p m	0	0	−		Flaccid

normal respiration is apparently essential for transport of auxin into the section (33) The absence of any inhibiting effect on streaming suggests that transport does not take place in the streaming protoplasm Similarly, Schumacher (265) could observe protoplasmic cyclosis going on simultaneously with *polar* movement of fluorescein in the cells of the cucurbit hair

As will be shown in the following section curvatures induced by gravity involve a movement of auxin laterally across the coleoptile or stem Here also it has been thought that an electrical gradient resulting from gravity, might be responsible, and long ago Brauner (47,48) showed that indeed the under side of a stem placed horizontal becomes electro positive to the upper side (the "gecelectric effect") The potential difference due to gravity is established before any curvature occurs, and there are several very suggestive relations between the potential and the subsequent auxin transport brought out by Schrank (264) No causal relationship has as yet been established however

It can only be concluded that auxin transport is not directly related to electric polarity; it is in some way related to respiratory processes but the link can readily be broken without damaging these processes

B Upward Transport

There are two conditions under which auxin is transported upward, *i e*, from base to apex The first is when it is applied to the upward-moving transpiration stream, as by pouring a solution on the soil (137) or adding auxin to a nutrient solution in which stem cuttings (138) or roots (272) are immersed In such cases, so long as transpiration occurs, the auxin is passively carried upward in the xylem in the same way as salts or dyes and the amount absorbed parallels the absorption of water It is a function of the transpiration rate but is also influenced by the concentration of salts in solution Skoog has, however, shown (272) in extensive experiments with tomato stems that auxin taken up in this way then moves laterally into the surrounding living tissues and is re-exported downward by the normal polar transport.

The other condition is when very high concentrations are applied Went and White (361), taking every precaution to avoid leakage along surfaces, still obtained inverse transport in the coleoptile when concentrations of 1000 mg /l indoleacetic acid were used Snow (282, see also 284) obtained curvatures apical to the point of application by using fairly high concentrations in lanolin, the effect was more marked when the application was close to the vascular bundle, so that it probably involved movement in the transpiration stream also Stewart (298) showed by *Avena* tests that auxin moved upward when very strong (2%) paste was applied to the first internode of a young bean plant It is probable that these effects are due to the toxicity of high auxin concentrations

Finally mention may be made of the interesting case of inverted cuttings, *i e*, cuttings rooted at the apex, budding from the base, and planted inversely In such cuttings there is a gradual development of a new series of cells from the shoots to the roots, opposite in polarity to those originally present, and correspondingly Went (357) found that at first the auxin transport is apex-to-base polar, but gradually base-to-apex transport appears as well Normal cuttings show no such change This phenomenon only serves to emphasize the strictly polar nature of auxin transport under normal physiological conditions

V. Role of Auxin in Tropisms

Although it was through tropisms that the role of the "growth substance" was first discovered (see Section I) interest in the past ten years

has shifted away from this aspect The majority of the facts have been discussed in detail by Went and Thimann (360), for phototropism the older literature is treated *in extenso* by DuBuy and Nuernbergk (56) and more recent summaries are given by van Overbeek (231) and by Oppenoorth (225) Only the briefest outline will therefore be given here

A Geotropism

Geotropism is the curvature of shoots away from the earth (negative) or of roots toward it (positive) The latter is not well understood because the role of auxin in the growth of roots is not clear The former, however, is explained satisfactorily by the Cholodny-Went theory,[5] namely, that when a shoot is horizontal more auxin moves to the lower side than to the upper, the lower side therefore grows more, causing upward curvature (Cholodny, 60) First worked out by Dolk (78) for coleoptiles by allowing the auxin from upper and lower halves to diffuse into two separate agar blocks, this experimental analysis of geotropism has since been generally accepted for all growing shoots, it has been confirmed by several workers (45,76) and with both extraction and diffusion methods Incidentally it provides one of the best illustrations of the strict limitation of growth by auxin supply instead of the two halves each receiving 50% of the available auxin the lower receives some 65–70%, and this difference is sufficient to cause immediate geotropic curvature

Gravity does not of itself cause any increase in the total growth rate ("geogrowth" reaction) (78) nor in the auxin production rate (76) or total auxin content, except in the mature nodes of grasses, which when placed horizontal begin to form auxin afresh (261), the same phenomenon occurs in sugar cane (234) and is apparently due to the liberation of free auxin from a bound form It is worth noting that "lazy" maize, which is insensitive to gravity and grows horizontal, does not show the normal accumulation of auxin on its lower side but accumulates a slight excess about 55%) on the upper side, as shown by van Overbeek (229) and Shafer (270), many other prostrate and "lazy" plants, however, show normal geotropic response (185) (see p 34) Another interesting exception is furnished by the action of ethylene, which causes positive geotropism in shoots of *Vicia*, here an excess of auxin accumulates on the upper side instead of the lower (178) so that ethylene must influence the transverse transport of auxin, a phenomenon extensively studied by Borgström (36)

It should be added that the auxin transported laterally is only the free-moving auxin of the coleoptile This was made clear from Went's studies (358) of the relation between diffusible and extractable auxin in

[5] So called because it was proposed by Cholodny and confirmed by Went

regard to growth and tropisms After decapitation, the geotropic sensitivity falls to very low values (78) and does not reappear again until new auxin production occurs ("regeneration") 2 5 hours later. The total extractable auxin, however, only falls to about 50% of the initial value before regeneration sets in On the other hand, the free-moving auxin, determined by diffusion out of the tip, falls, like the geotropic sensitivity, almost to zero, until regeneration starts. Thus it is the diffusible auxin which is redistributed by gravity

The mechanism by which auxin is transported laterally under the influence of gravity is unknown Attempts to correlate it with "geoelectric potentials" have been without success, as discussed in Section IV for normal transport It would seem that gravity can only be perceived by something falling; the older literature ascribed much importance to small starch grains, the "statoliths" of Haberlandt, but as yet no relation between the movement of these and the movement of auxin has been established

B Phototropism

Phototropic curvatures are more complex, since they vary both quantitatively and qualitatively with light intensity. In the *Avena* coleoptile, which has been most studied, curvature takes place toward the light (positive phototropism) under low light quantities, away from it (negative) at higher, and toward it again at still higher. For the first positive curvature (at 20–100 meter candle seconds), Went showed in 1928 that more auxin diffuses from the dark side of the tip than from the lighted side Similarly, for the negative curvature (at 1400 meter candle seconds), Asana found more auxin diffusing from the light side of the tip (11). These results suggest the simple Cholodny-Went theory, namely, that light causes lateral movement of the auxin which is responsible for the curvature They explain the earlier experiment of Boysen-Jensen (43), who divided the coleoptile tip longitudinally with a fragment of glass, when this was done parallel to the direction of the light, curvature took place, but, when perpendicular to the direction of the light, curvature was prevented, presumably by stopping the lateral transport Further, the same lateral transport to the dark side was found in seedlings of two dicotyledons *Raphanus* by diffusion (226) and *Phaseolus* by extraction with chloroform (45) Light does not affect the normal longitudinal transport of auxin (226,299a, but *cf.* 55a)

However, there is another effect, namely, that a given amount of auxin produces more growth in the dark than in the light (226,331) Insofar as low light intensities are concerned, this appears to be due to a destruction of auxin—probably auxin *a* (166)—by light In his original

"redistribution" experiments Went (348) found by diffusion less total auxin (dark and light sides combined) after illumination than in dark controls, and this was confirmed with the ether extraction method, both by Stewart and Went (299a) and by Oppenoorth (225) The extent of inactivation does not seem to increase very much with time of exposure, at least as far as the data go, one second of sunlight caused about as much inactivation as sixty seconds (299a) The destruction is of the order of 25% and is accompanied or followed by the shifting of the auxin toward the dark side (55a,225) Longer exposures cause an increased synthesis of auxin (225), which is discussed below

The mechanism of this effect has been extensively studied by Kögl and colleagues at Utrecht Koningsberger (173) found in 1936 that auxin *a* lactone shows ultraviolet absorption due to its very rapid conversion to an inactive product, "pseudo-auxone", even the weak irradiation needed to determine its ultraviolet absorption spectrum inactivates 80–100% of the auxin activity (161) Since the free acid (auxin *a*) and its lactone are in equilibrium in weakly acid solution and since only very weak light is necessary,[6] there is here a mechanism for inactivation by light What is more important is that the inactivaction may occur in the visible spectrum through the mediation of suspensions of carotene (170,266) Both α- and β-carotenes and some other carotenoids are effective Since carotene is present in the coleoptile (343) and particularly in the apical two millimeters (52), it can hardly be doubted that through this system auxin *a* is destroyed *in situ* by light Further, the spectral sensitivity of the coleoptile to light (19,148) agrees well with the absorption spectrum of a carotenoid This is, then, a second mechanism for phototropic curvature

There are two further points in regard to photoinactivation The first is that in the light-sensitive sporangiophores of certain fungi, *Phycomyces* and *Pilobolus*, the curvature also follows the carotene absorption (52,58) and a small part at least of the auxin present is auxin *a* (172) These facts and the presence of carotene, demonstrated by Bünning (52) indicate that here also curvature might be due to photoinactivation of auxin *a* lactone sensitized by carotene Indeed, Kogl and Verkaaik (172) have no hesitation in drawing this conclusion, although undoubtedly most of the auxin of *Phycomyces* is indoleacetic acid, as was shown first by the diffusion constant determinations of Heyn (134) Furthermore, we have as yet no evidence that the growth of fungal hyphae is controlled by auxin Hence this explanation for phototropism in the fungi needs far more support

[6] The "quantum yield" is stated to be very high—of the order of a million or more (170)

The second is that, in green plants exposed to the relatively high intensities of daylight, even indoleacetic acid produces less growth than in the dark, as shown by Thimann and Skoog (331) Elongation of all plant stems is, of course, reduced by bright light, and indoleacetic acid as we have seen above, occurs widely as an auxin As yet, there is not much quantitative information known about the photoinactivation of this substance, though in solution it does suffer a rather slow light-accelerated decomposition (Algeus, (5)) [7] In crude plant extracts, which contain traces of carotene, it is rapidly inactivated by sunlight (187), and the same is true when indoleacetic acid is dissolved in agar It is therefore entirely possible that phototropism may be mediated by indoleacetic acid and is not as formerly supposed, dependent on auxin *a*

Finally the effect of light on auxin synthesis must be mentioned All plants studied form more auxin in light than in dark (213 331), and on placing in complete darkness auxin rapidly disappears (see discussions in Went and Thimann, 360 Chapter 4, and in Boysen-Jensen 46 Chapter 4 Oppenoorth (225) has, however, found that an increased synthesis appears within a few minutes after illumination of coleoptiles with moderately high intensities (3000–26000 ergs/cm ^{2}), and considers that the negative curvature and the second positive curvature are largely due to differences in auxin synthesis on the two sides The increased auxin produced, insofar as it is auxin *a*, will of course equilibrate with its lactone and then be inactivated by light, and no doubt under long exposure, or continuous illumination, the two processes will keep pace On the other hand the increase may well be due to indoleacetic acid, for Larsen (187) found that when etiolated seedlings are exposed to light the (presumptive) indoleacetaldehyde decreases and acid auxin increases This simple oxidation might account for such a rapid rate of formation of auxin

A number of plants particularly among the grasses, grow prostrate in the field and Langham (185) has shown that in many of them this behavior is due to negative phototropism in sunlight, while in weaker light intensities they show normal positive phototropism In connection with Asana's work mentioned above, an auxin analysis of these would be very valuable It is important to note that "laziness" may thus be due to interference either with geotropism or phototropism (see p 31)

C Other Tropisms

The geotropism of roots seems to agree with the Cholodny Went theory Root elongation is inhibited by auxin, except in the very lowest

[7] The paper of Algeus contains an excellent discussion of the effect of auxin on unicellular algae

concentrations (Section VII, B), and correspondingly there is good evidence that when roots are placed horizontal auxin accumulates on the lower side, reducing growth there and thus causing downward (positive) curvature Traumatotropism, or curvature toward a wound, is due to two factors the wound interferes with the transport of auxin, and enzymes set free by the killed cells rapidly inactivate auxin by oxidation Both processes act in the same direction, *i e*, to cause less growth on the wounded side Other tropisms have been as yet insufficiently studied A fuller discussion of tropisms will be found in ref 360 (Chap 10)

VI Root Formation

The formation of roots on pieces of stem or "cuttings" was studied by early physiologists as a parallel case to the regeneration of organs in invertebrates However, while the problems of regeneration are almost as obscure now as they were at the turn of the century, the nature of root formation has been considerably elucidated, mainly through the discovery of the role of auxin

A Auxin as a Root-Forming Hormone

The idea of an internal factor or hormone which controls rooting was first brought out by van der Lek (191) who showed that when preformed root initials are not present, new roots are formed strictly at the base of a stem section buds on the stem promote formation of roots below them and if the cortex below the bud is removed this effect is prevented Thus he postulated a root forming hormone produced by buds and travelling downward in the phloem (see Section IV) Following his work on auxin in the coleoptile, Went (349) showed that a diffusate from leaves, applied to the apex of a cutting increased the number of roots formed, and Bouillenne and Went (40) then found that diastase and rice polishings extract were effective These workers also found that application of sugar increases the number of roots formed, and they distinguished between its nutritive effect and the effect of the hormone, which is transported in a polar direction from apex to base only The distinction between nutrients, stored in cotyledons etc, and special root-forming substances was also brought out by Němeć (215), whose ideas, developed independently, are similar to those of Bouillenne and Went in some respects

Using the standard test of Went (350) with stem sections from etiolated pea seedlings, Thimann and Went (335) began the isolation of the root forming hormone but soon found that the richest sources were materials like *Rhizopus* medium and urine extracts (see p 16) which

were rich in auxin, the root forming activity accompanied the auxin activity through extraction with various solvents and all purification stages and the chemical properties of the two hormones appeared to be identical The identity was finally proved a few months later in two laboratories when synthetic indoleacetic acid was shown to have high activity for root formation on pea stems (Thimann and Koepfli 323) and purified auxin *a* on *Tradescantia* stems (Kögl 158) the latter plant material had just previously been shown to produce roots when treated with extracts of urine or pollen by Laibach Müller and Schafer (183) (see also 181)

The discovery that root formation on cuttings is induced by auxin and the availability of synthetic auxins have led to a vast amount of work on the application of this technique in horticultural practice The rooting of cuttings is one of the main practical methods of propagation of course and much of the literature deals with conditions and concentrations of auxin most suitable for particular plants An excellent review and a long table of results arranged by plant species and variety has been published by Pearse (240) and another long group of tables by Mitchell and Rice (205) a still more complete listing has just appeared (317a)

B Substances Active

In general all substances which have growth promoting activity in one of the standard tests (see Section II) appear to be active in root formation After indoleacetic acid and auxin *a* and *b* had been shown to be active indolepropionic acid very weakly so and indolecarboxylic acid quite inactive (323) Zimmerman and Wilcoxon (376) added α naphthaleneacetic indolebutyric phenylacetic and fluoreneacetic acids in approximately that order of effectiveness Thimann (311) added indeneacetic and coumarane 2 acetic acids and showed that these two substances are poorly transported but are fully active when applied to the base of the internode where the roots were produced There is some uncertainty with phenylacetic acid which appears to have no true root-forming activity and yet to be an auxin in other respects (354) Phenoxy and napthoxyacetic acids and their chlorinated derivatives also naphthyl acetamide naphthylmethylsulfonic acid and 4 methylthiazole-5-acetic acid (339) are all active The esters of some of these are almost as active as the acids and being volatile can be applied to the whole plant in vapor form Veldstra (339) has tabulated the relative activities of a great many substances for root formation

C Interactions between Factors

It is a peculiar fact that the combination of two auxins will sometimes produce more roots per cutting than one acting alone This was first

shown for the combination of auxin *a* with indoleacetic acid (360, p 195) and later for indoleacetic with naphthaleneacetic acid (9), for indoleacetic with phenylacetic acid (354), and for indolebutyric with naphthaleneacetic acid (139) Such effects are hard to explain, since it seems unlikely that each auxin can exert a fundamentally different effect and that these can then be summated In the *Avena* test a weak auxin may actually inhibit the action of a stronger one (272a) It might be, of course, that certain cells or tissues enzymically destroy one auxin rather than another so that a single auxin cannot be effective on all tissues Went (354) considers that root formation involves two processes, the first of which can be carried out by substances which are not necessarily auxin-active ("hemiauxins") while the second requires a true auxin, his experiments used successive treatments rather than mixtures

The combined action of auxin and nutrients is more readily understood, for the formation of roots and their subsequent growth involves the laying down of cell walls and synthesis of protoplasm Treatment with sugar, particularly with etiolated cuttings deprived of food reserves, often promotes rooting (40 350), but even woody cuttings (83,236 321, 322) are often benefited Since cuttings are essentially starved during the ordinary process of rooting in the nursery bench, other nutrients are sometimes also effective Complete nutrient solutions (21,107 325) may be used, but the calcium and magnesium may have inhibiting effects (325), and it seems rather that the principal constituents needed are nitrogenous, especially nitrate or ammonium, and adenine or other purines (77,236 325) The supply of organic nitrogen and of carbohydrates probably accounts for the favorable effect of leaves on cuttings which is often proportional to the number of leaves present (144,248), indeed the effect of the leaves can be duplicated by a suitable combination of sucrose and nitrogen (236)

The growth of isolated roots in culture solutions *in vitro* is dependent upon thiamin (see following chapter), and while it might be thought that the minute amounts of thiamin needed for root growth on cuttings could be supplied by the stem nevertheless thiamin does promote rooting of some cuttings (322,344) or subsequent growth after rooting (240) Other members of the vitamin B complex may be mentioned, biotin has a large effect on etiolated pea cuttings in auxin plus sugar (360, Chapter 11) which has not been reported for other plants, while nicotinic acid and choline (236 325) are also favorable The role of an additional hormone-like substance, "rhizocaline" will be discussed in Chapter III

D Anatomical Studies

The nineteenth century botanists such as van Tieghem were much concerned with the specific tissues from which roots arose However

the auxin work appears to show that root initials may be produced in almost any living tissue They have been reported in epidermis pericycle, endodermis, cortical parenchyma, and even in pith, particularly by Dorn (79), Kraus *et al* (175), and Hamner and Kraus (129) In this sense plant tissues approach the "totipotency" of the animal embryologists In line with the older views, however, roots do seem to arise more frequently from the pericycle than elsewhere (128)

E Methods of Treatment

Root formation on cuttings can, of course, be induced by application of auxin at the apical end, its polarity of transport leading to rapid accumulation at the base However, as mentioned in Section IV, the capacity for transport is limited so that, when high concentrations are applied to the apex roots will be formed there also Conversely, when high concentrations are applied to the base, roots are formed there only Since many active substances are only poorly transported also, the logical procedure is to apply to the base Concentrations from 0 25 mg /l for sensitive herbs up to 200 mg /l for resistant woody plants applied for 24 hours to the base, are used in practice A few seconds' dip in highly concentrated (several grams per liter) alcoholic solutions is a practical alternative The cuttings may instead be dipped in talc containing the auxin enough adhering to the moist surface for effective action Auxin may also be applied in lanolin paste almost anywhere on the cutting, this application is sometimes made a few days before the cutting is removed from the plant Removal of epidermis, or even of the whole cortex or splitting of the cuttings at the base, greatly facilitates auxin uptake in some species (*e g*, Hubert *et al*, 145) The resulting increase in rooting may however, be partly due to the wound stimulus Uptake of the solution is favored by high transpiration or by partial drying of the cuttings beforehand (335a)

VII Phenomena of Inhibition and Toxicity

One of the most curious features of the physiology of the auxins is that while they promote so many growth processes, they also have growth inhibiting effects Two of the most marked of these are the inhibitions exerted on the development of buds and on the elongation of roots The inhibition of the development of an abscission layer at the base of petioles and fruitstalks has many features in common with bud inhibition Because the subject has been extensively reviewed (316) and because more recent work has thrown little fundamental light on the phenomena a brief recapitulation here will suffice In addition the general toxicity of the auxins, a subject with no direct bearing on the

normal hormone physiology of plants, will be discussed briefly because of its important applications to agriculture

A Bud Inhibition

1 *The Facts*

In dicotyledonous plants the stem apex is a terminal bud This bud normally produces auxin, mainly from the young developing leaves in it (12,17,331, see also 279), but also to some extent from the stem apex itself (101), and this auxin promotes the development of the stem immediately below it However, the same auxin also prevents the development of lateral buds lower down on the stem thus allowing the terminal bud to retain its "apical dominance' When the terminal bud is removed, as in pruning, one or more lateral buds (usually those in the most apical axils remaining) begin to develop, in so doing they also begin to produce auxin, which in turn inhibits the buds still lower down If, after removal of the terminal bud, auxin is applied in its place, the lateral buds remain inhibited

The first demonstration that bud inhibition is due to a diffusible substance was made by Snow (278), who showed that the inhibiting influence coming from the terminal bud in *Vicia faba* could cross a discontinuity of tissue, this experiment corresponds with those of Boysen-Jensen and Paál for the promotion of growth (see Section I) Eight years later Thimann and Skoog confirmed Snow's finding and identified the inhibiting influence with auxin, which at first (330,331) was obtained from *Rhizopus*, and later (273) with pure indoleacetic acid and auxin *b* Laibach also showed that an inhibiting substance diffused from pollen (180) Confirmation with numerous different plants soon followed (73,81,101,136,337) The concentrations needed for inhibition, though somewhat higher than for growth, are entirely physiological and not toxic, for lateral buds which have been inhibited in this way resume their growth when the auxin source is removed (331) Several different natural and synthetic auxins have been shown to be effective (117,136, 176,273 311) It should be mentioned that leaves also exert an inhibition, though to a lesser extent than the terminal bud, as was shown earlier by Dostál (80)

2 *Mechanisms*

The way in which the inhibition is brought about is far from clear The many hypotheses have been reviewed by Snow (283) and Thimann (316) At first it was thought that the auxin at the apex (either produced naturally by the bud, or applied artificially after decapitation) in some

way diverted to itself substances necessary for bud growth and thus starved the lateral buds (211,354; see also the discussion in Section V of the following chapter). This is similar to the view of Goebel and other older botanists who considered that a growing apical bud *maintained its* dominance by using up the available nutrients A modification of this view is that of Ferman (85), who suggested that the growing bud draws to itself the supply of auxin precursor, so that the laterals are unable to produce auxin This is supported by the undoubted fact that inhibited buds produce less auxin than do growing buds (331) and, though the evidence is not quite consistent, they also appear to contain less total extractable auxin than growing buds (85,228). In other words, there is some reason to think that the inhibition is exerted not so much on the growth of the bud as on its ability to produce auxin

However, it now seems clear that inhibition cannot be primarily an indirect effect due to the diversion of materials away from the bud, since application of auxin directly to the lateral buds, either *in situ* on the stem, as in the experiments of Pleh (241) and Thimann (314), or isolated and growing in nutrient solution, as by Skoog (272), causes clear-cut inhibition of their growth Also in small fragments of plant tissue in culture, particularly root tissues, auxins such as naphthaleneacetic acid strongly inhibit the development of buds (96,98) In slices of potato tuber the local application of auxin inhibits bud development without producing any corresponding growth elsewhere (81,202a) Exposure of whole potatoes to auxins in vapor form (*i e*, methyl esters of the acids) causes inhibition of all the buds (123) In none of these cases would it seem that the effect can rest on movement of materials elsewhere; the effect is primarily local

It appears that the influence of auxin on different organs is represented by a series of optimum curves, intermediate concentrations promoting growth and higher concentrations inhibiting it (45,314), as shown in Fig 7 (p 45) Thus the concentrations causing stem growth would be high enough to inhibit bud development. This general theory receives support from the numerous effects of auxin in Gautheret's cultures of various organs (96, see Section VIII, A), and additional considerations which may help to explain it have been advanced by Skoog *et al* (272a); against it is the lack of any cambial activity in inhibited buds (280) although auxin is known to stimulate the cambium (see Section VIII). A peculiar and unexplained fact is recorded by Castan (57), namely, that, if high auxin concentration is applied to the intact terminal bud, it loses its power of inhibiting the lateral buds below it.

The problem is made more complicated by the direction in which inhibition is exerted Since auxin moves polarly from apex to base,

inhibition should be only exerted on buds morphologically below, *i e*, basal to, the source of auxin Although this in general is strictly the case, there are exceptions in which buds are inhibited above the point of auxin application (283,285), and a parallel has been suggested with certain upward inhibitions of stem elongation, studied by Pohl (243), Le Fanu (190), and Mitchell and Martin (204) The phenomena of geotropism, however, provide clear agreement with expectation, here the auxin is known to be diverted to the lower side of stems by gravity, so that we should expect to find that in horizontal stems the buds on the lower side are inhibited, this was observed as long ago as 1917 by Loeb and has been confirmed in different plants by many workers (73 237,249,272)

One type of phenomenon which might have significance for bud inhibition has not yet been brought into the picture Many workers have found evidence for growth-inhibiting material in plant tissues, particularly in ether extracts thereof Kockemann (154 155) extracted such material from fruits, demonstrating its effect by inhibiting the germination of seeds This so-called 'blastokolin' was investigated by Kuhn *et al* (177), who extracted an oil from *Sorbus* fruits which strongly inhibited seed germination, and demonstrated that parasorbic acid had similar effects Other substances having an unsaturated lactone structure (340), including coumarin act in the same way Moreover, Voss (342) extracted from corn, and Larsen (186) from tomatoes, material which inhibits growth of the *Avena* coleoptile Linser (199) made similar extracts from lilac leaves and showed that they also inhibit the formation of roots Juel (152), in an extension of Larsen's work developed an assay method by mixing the inhibiting extract with known concentrations of auxin in agar and using the *Avena* test on the mixture She showed that the inhibition is not due to auxin inactivation, and that it is exerted also on root growth which itself is inhibited by auxin (see pp 43–46) Hence the extracted material is not simply an antiauxin, but an inhibitor of the growth of both shoots and roots Similar experiments have been carried out with sugar cane nodes, from which the inhibitor is liberated by hot water (237,237a) The dormancy of potato buds has been shown by Hemberg (131a) to be due to an inhibitor present in large amounts in the periderm, and disappearing slowly as the tubers mature The auxin content does not change during dormancy but increases shortly before sprouting

More suggestive still is the inhibitor of Stewart *et al* (297,299), which produces a marked positive curvature (*i e*, toward the block) in the *Avena* test This substance was partially purified and shown to yield an auxin—most probably indoleacetic acid—on alkaline hydrolysis If it could be shown that lateral buds have the property of producing this

inhibitor directly from auxin, a mechanism of bud inhibition would be at hand Although as yet there is no such direct evidence, the scheme advanced by Skoog *et al* (272a) gives a very plausible rationale for this *Furthermore, Snow* (280) has brought forward independent evidence that bud inhibition is due to a special inhibiting hormone in some way produced by auxin This concept has recently been discussed by Skoog (274a)

The situation can be summed up by saying that most of the data point to bud inhibition as due to auxin directly, with the mechanism probably involving the formation of an inhibitor by or under the influence of auxin The possibility is not excluded, however, that other substances necessary for growth may in some way play a part

3 *General Significance*

The inhibition of one bud by another is a phenomenon of very wide occurrence and has a broad influence on general morphology In tubers, for instance, development of a bud at the apical end leads to inhibition of others but ringing or physical isolation of these buds, allows the lower buds to develop (81,131,202a) The auxin is presumably carried from one bud to another through the cortex Auxin application, either as paste to the outer cortex or as vapor to the whole tuber, maintains the buds in the inhibited state and this is now being used on a large scale in the storage of potatoes with methylnaphthalene acetate It is of interest that such auxin inhibited buds resemble normal dormant buds in that they are stimulated to sprout by ethylene chlorhydrin (123 202a) This treatment greatly increases the rate of auxin destruction, thus releasing the buds from inhibition, when growth begins again the terminal bud soon re-establishes its inhibition of the laterals again through the auxin mechanism (202a) It is not free auxin itself, however, but a specific inhibitor (131a) which is responsible for the absence of bud development during the dormant period

In general the tall rapidly growing single shoot type of plant, which presumably produces and transports auxin efficiently, has few lateral branches while shorter dwarf or stunted forms typically become bushy with numerous laterals or tillers Auxin relations of this sort have been studied by van Overbeek (227 230) and Delisle (73) but much still remains to be done Young leaves since they are potent sources of auxin exert a powerful inhibition (279) but mature leaves also inhibit in some plants (80) In guayule a desert composite grown for its rubber content the mature leaves actually inhibit the buds in their axils more *powerfully than does the terminal* bud (277) Indeed, a single leaf can inhibit the lateral buds all the way down the stem, a most unusual behav-

ior, which may well repay closer study In *Solidago* plants in the rosette stage, each leaf inhibits somewhat the growth and development of the next, a phenomenon presumably parallel to that of bud inhibition (101)

In the ferns, Albaum (1) has brought to light a parallel situation, the heart-shaped prothallia respond to the removal of their growing apex by formation of a new outgrowth (of the same shape as the indented area which they replace), and this "regeneration" can be inhibited by applying auxin in lanolin Similarly, if the young sporophyte which develops later out of the prothallium is removed, another grows in its place, while application of auxin to the cut stump prevents this These phenomena are thus quite parallel to the inhibition of buds, although buds as such are not involved Doubtless Nature has provided many similar variations on the same theme

B Root Inhibition

Besides simple growth promotion, the first additional effect of auxin to be discovered was the inhibition of the elongation of roots This was when Nielsen (217) extracted a crude auxin from cultures of the fungus *Rhizopus suinus* and showed that it promoted growth of the coleoptile but inhibited that of the root The experiments were repeated and extended by Boysen-Jensen (44) and Navez (214) and finally done with pure auxins by Kogl, Haagen Smit, and Erxleben The technique is simply to immerse the roots of young seedlings in serial dilutions of the auxin and measure elongation with a millimeter scale There is some thickening, but this is not, as was first thought, sufficient to compensate for the decrease in length, the auxin therefore produces a large total decrease in root weight (312) The inhibition in length is roughly proportional to the logarithm of the concentration, so that the effect has been used as a simple auxin assay by Lane (184) and Bonner and Koepfli (34) Control of pH is essential (202), since auxin enters tissues much more readily in the free acid form than as an ionized salt (4,326) The activities of a number of substances have been compared in this way (92,184,202,311, especially 34) and it appears that, in general, compounds which have auxin activity as measured by growth promotion also cause root inhibition, if inactive in the *Avena* or pea test they are inactive in inhibiting roots The inactivity of indolecarboxylic, α,α-dimethyltoluic and *trans* cinnamic acids (34) is of particular interest in connection with the relation between structure and activity discussed in Section III, C Recently Thompson *et al* (336) have published this as a new method, and tested 1060 compounds with it Of these, the most active were 2 4-dichlorophenoxyacetic acid, its anhydride, sulfoamide, and certain esters,

2-methyl-4-chlorophenoxyacetic acid, its anhydride, amide, some esters, and other derivatives, 2 bromo-4-chlorophenoxyacetic acid, and 2 methyl-4-fluorophenoxyacetic acid The first-mentioned is highly active in the curvature of slit pea stems (see Section II, D), though it gives only minute curvatures on *Avena* coleoptiles Doubtless all these substances will be found to show growth-promoting activity on one or other of the standard growth-promoting auxin assays

Of course, an inhibition is less specific than a growth promotion, and many compounds have some inhibiting effect in relatively high concentrations For this reason the inhibition of germination, studied by soaking seeds in solution and termed the "blastokolin" test (see Section A above) may not be very specific, it appears, however, to have no relation to the inhibition of root elongation by auxin For instance, the ether-soluble growth inhibitor of tomatoes inhibits both root and shoot growth (152) It is well known, too, that colchicine inhibits root elongation and causes characteristic swellings just proximal to the root tip (see, *e g*, 82 192 201) It is perhaps remarkable that the changes in electric potential differences along the root which are caused by colchicine treatment are very similar to those caused by indoleacetic acid (338) This does not, of course, necessarily mean that, as Umrath and Weber (338) suggest, colchicine produces its effect by "activating" auxin in the root, for its effect on mitosis is far stronger than that of auxin However, it is at least suggestive that the swellings induced by auxin in roots were shown by Levan (193) to contain many polyploid cells

In contrast to the inhibition, extremely low auxin concentrations cause slight acceleration of root growth This was discovered independently by a number of workers in 1936 (7,8,54 84,86 99,312), only Jost and Reiss (151) could find no acceleration The effects are small but real indoleacetic acid at $10^{-9}M$ causes about 30% acceleration The response of roots to auxin is thus given by an optimum curve with its peak at excessively low auxin concentrations, as shown in Fig 7 Also if the inhibition is not too great, it is accompanied by the formation of lateral roots *i e* by branching (151,312 372) The same effect results from decapitation of the root tip However, such branching is not simply due to the inhibition of the tip growth, but is directly caused by auxin because as shown by Thimann (311), when auxin is applied to the base of the stem of *Pisum*, it slightly accelerates the growth rate of the main root, but still promotes the formation of laterals

Short exposure of roots to auxin causes a temporary inhibition followed by a stimulation which may lead to a general stimulation of growth of the entire plant (92 324) This "after-effect" is probably the cause

of the accelerated growth of "hormonized" seeds first reported by Cholodny with oats in 1936, and discussed further on p 54 The duration of the inhibition is proportional to the time of exposure to the auxin and Gast (92) has shown that the amount of stimulation which follows is roughly proportional to the amount of inhibition A detailed analysis of the phenomena of root elongation will be found in the papers of Burstrom (54) He divides the process into a phase of increasing elasticity, which is accelerated by auxin, and one of decreasing elasticity (during which most of the growth takes place), which is inhibited by auxin

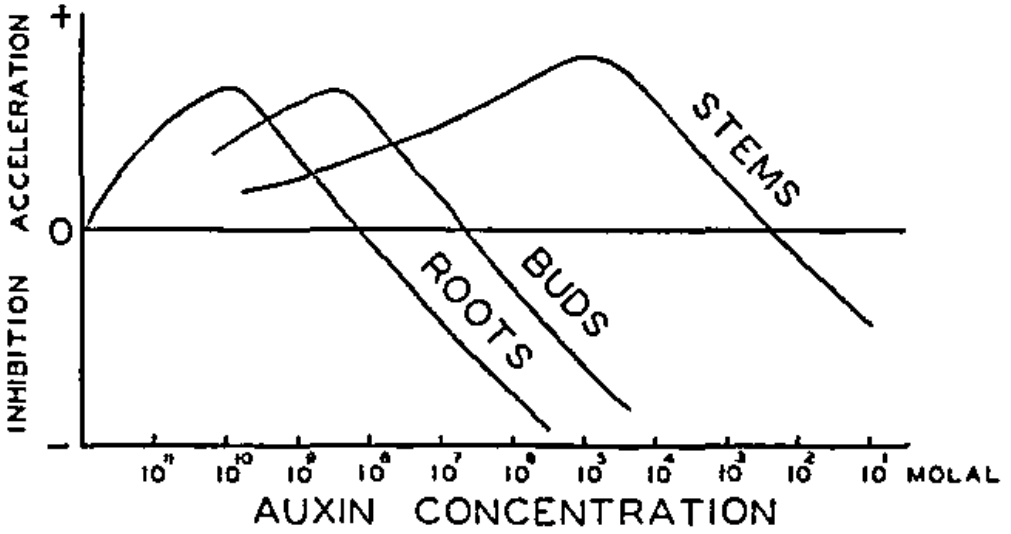


FIG 7 —Diagram of the inhibition and growth promotion of different organs as a function of auxin concentration The abscissae for the bud and stem curves are only approximate (From Thimann, 314)

The effect of auxin in inhibiting root elongation acquired special interest as an explanation of the geotropism of roots (see 360, Chapter 9) This geotropism, which is positive, *i e* toward gravity, would thus be due to the accumulation of auxin on the lower side as in shoots, but with the difference that the auxin would cause greater inhibition on the lower side This was the original Cholodny-Went theory of geotropism, but it has never been really rigidly established While all experiments point in this direction, the closeness of the growing zone to the tip in many roots has made it extremely hard to obtain clear-cut growth responses after decapitation The production of auxin by the root tip has also been hard to establish, in spite of many extraction and diffusion experiments (see especially 44,45 86 246,247,267) To sum up briefly many contradictory facts and interpretations (discussed by Fiedler, 86, and Thimann, 316), it appears clear now that small amounts of auxin are in fact regularly produced in the root tip provided it is adequately nourished (235,267) If this is to be enough so that its geotropic accumulation on

the lower side would account for positive curvature, it should also be enough to cause at least slight growth inhibition when the root is in the vertical position In other words decapitation should cause slight acceleration of root growth Some investigators have indeed found this effect, but agreement is not complete, perhaps due to the morphological difficulty mentioned above which makes the length of the tip cut off extremely critical It should be noted, too, that exposure to light increases the auxin content of isolated roots (267) and correspondingly inhibits elongation (253) Differences in lighting may thus also account for lack of agreement among different investigators

Since high auxin concentrations also inhibit elongation of stems it might be supposed that stems supplied with considerably more auxin than they receive under physiological conditions should show positive, *i e*, downward geotropism This has been claimed, indeed, by Geiger-Huber and Huber (100) with mustard seedlings, but it is more probable that the downward curvature reported is not due to growth, but merely to plastic sagging since Burkholder (53) has shown that similar downward curvatures are prevented by balancing the weight of the shoot

C Inhibition of Abscission

The falling of leaves and mature fruits is due to the formation of an "abscission layer' of cells across the base of the petiole or fruitstalk, and to the separation of the walls of these cells from one another In experiments with *Coleus* Laibach (180) found that this abscission is prevented by applying orchid pollinia to the petiole The phenomenon was discovered independently by La Rue (188) and shown to be produced by several pollens and leaf diffusates and also by pure auxin (indoleacetic acid) *Coleus* is convenient for these experiments because the petioles fall quickly when the blades are cut off, *Ricinus* and *Bryophyllum* behave similarly The reaction is simple and by its means Gardner and Cooper (89) have compared the activity of nine auxins and shown that 156 other compounds without auxin activity do not delay abscission

The interest in this phenomenon lies primarily in its application to fruitstalks which often abscise before the fruit is completely mature Gardner and Marth (91) and Hoffman *et al* (141) showed that the premature dropping of apples can be conveniently delayed by auxin treatment Spraying or dusting with auxin in early September will delay fruit drop at least two weeks This procedure is now widely used by orchardists, directions for its use have been given by many experiment stations

Falling of the needles of evergreens at least in *Tsuga* (335a) and *Taxus* (81a) is also delayed by auxin in *Taxus* a concentrated nutrient solution acts in the same way

D General Toxicity

It has been known for many years that high auxin concentrations are toxic This was first noted in experiments with plant parts immersed in solutions for growth measurements (30,326,334), with whole plants treated with auxin solutions (106), and with cuttings treated with auxin solutions at the base (by many workers, see 360, p 204) In concentrations just below the toxic level, growth inhibition commonly occurs (see discussion in 316) and inhibitions may be caused above a local application of auxin to the stem (190,207,242,282) Further, as discussed above, root growth is powerfully inhibited by auxins The toxic effects, as opposed to mere inhibition, have been recently put to practical use In parallel experiments in the United States and England, it has been shown that simple spraying with relatively high concentrations of auxins (about 1000 mg /l) will kill many dicotyledonous plants The most effective substances are those which are of high auxin activity[8] and stable against soil microorganisms, particularly 2,4-dichlorophenoxyacetic acid and related compounds (22,27,127,223 275,276,308,336,345) Aqueous sprays of the free acid or its esters or salts appear to be the most effective Because the grasses and cereals are relatively insensitive, it is possible to exert what the English workers call "selective herbicidal activity," and destroy weeds in standing cereal crops This application is of very great agricultural importance and is already being used to eliminate such pests as ragweed, bindweed, and water hyacinth No attempt will be made here to discuss or even list the flood of papers on this topic in recent horticultural literature A recent review has been given by van Overbeek (234b)

The exact nature of the toxicity of auxins is not clear The killing of whole plants in soil may rest in small part on root inhibition, but usually involves complete rotting of the roots and rapid dying of the leaves Furthermore, toxicity is exerted on isolated stem or coleoptile sections in solution and indeed at concentrations as low as 50 mg /l Such objects show a clear optimum curve in their auxin response It is probably significant that many toxic substances cause stimulation at low concentrations, inhibition or toxicity at high Examples are the heavy metals, cyanide, 2,4 dinitrophenol, and iodoacetate However, in none of these instances has the change of sign of the effect been satisfactorily explained In popular literature it has been stated that the auxin weed killers cause plants to "grow themselves to death," but there is little basis for this statement

[8] Went (private communication) has, however, found that some substances inactive as true auxins are effective as weed killers

VIII Other Actions of Auxin

A Cell Division

1 *Tissue Cultures*

The phenomena of cell division in isolated fragments of plant tissue were first studied some forty years ago by Haberlandt in his classical but unsuccessful attempts to obtain plant tissue cultures The conditions leading to cell division in plant tissues are many and varied, but one of the main contributions of the work on tissue culture was to direct attention to the role of hormonal factors in the process The action of wound hormones and their possible interrelation with auxins in promoting cell division will be taken up in Chapter III, we will deal here only with the role of the auxins themselves in cell division

It is characteristic of roots that they grow well in culture media, with cell division keeping pace in a normal manner with cell enlargement, and without the necessity of adding any auxin There is no evidence that roots need any supply of auxin for their growth, though it is possible that they do produce small amounts of an auxin and that this suffices for their needs Much of the auxin in root tips disappears rapidly soon after separation from the plant (86) but small amounts remain, it is highly probable, though not rigidly proved, that there is a slow production of auxin by the tip even on a mineral medium (212 232,235) The total auxin of roots like that of many other tissues, is extractable with ether only very slowly (332), with *Avena* roots about three weeks of continual extractions are needed to reach a 75% yield The tumor cultures of White (363) also grow slowly without added auxin, but they definitely produce small amounts of it during growth (332)

In other instances cell multiplication appears to depend markedly on the presence of auxin This is well exemplified in tissue cultures While slices of carrot will develop for many transfers (with cell division) in a mineral medium containing only salts sugar, and a source of nitrogen, as was shown almost simultaneously by Nobécourt (218,219) and Gautheret (93 94) in 1937–1938 their cell division and growth are very greatly promoted by auxin at 1 mg /l Indeed, Nobécourt considers auxin essential for the carrot since its growth invariably stops after some months unless auxin is added This suggests either a very slow synthesis, or else a remarkable persistence of auxin in the tissue The tissue of Jerusalem artichoke (*Helianthus tuberosus*) develops *only* if auxin is added, indoleacetic or naphthaleneacetic acid at 0 1 to 1 0 mg /l are about equally effective For carrots, indolepropionic acid is ineffective (221) In such material the auxin behaves therefore as a cell division-

inducing substance Some cultures have been kept going in presence of traces of auxin, for over four years (Fig 8) Gautheret (95) points out that the new tissue formed in culture fragments is proportional to the amount of surface exposed which might suggest that wound hormones liberated at the cut surfaces also play a part (see Chapter III Section 1) The differentiation in the cultures seems not to be auxin controlled since it takes place also in carrot kohlrabi and endive cultures which do not at least at first require added auxin (97) Such conclusions however are uncertain until the formation of auxin in these cultures is examined

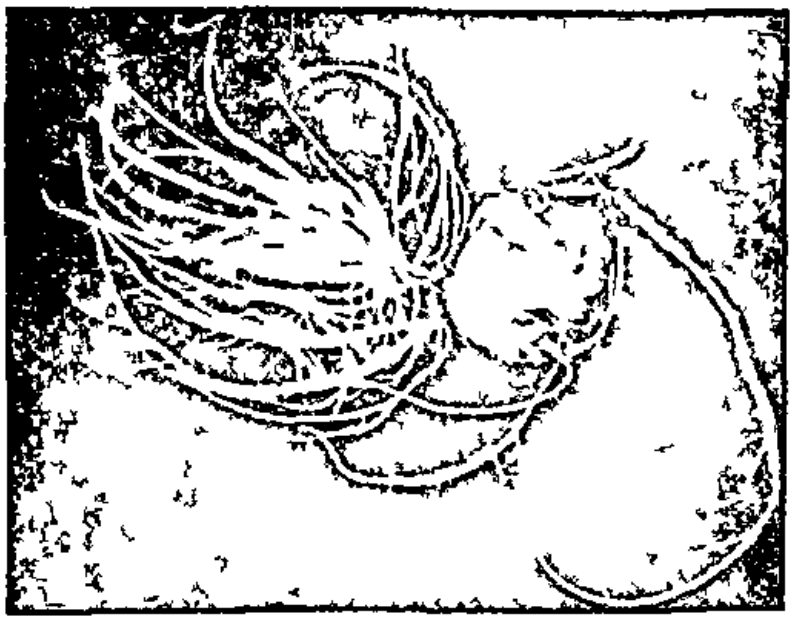


Fig 8 —Culture of endive tissue which has been maintained for over 4 years in presence of traces of auxin The fragment shown has grown for 28 days after the 25th transfer (From Gautheret 93)

Thiamin is certainly synthesized by the carrot cultures of Nobécourt (220)

The concentration series in the action of auxin here is of interest (96) With carrot endive and Jerusalem artichoke increasing concentrations of naphthaleneacetic acid produce in order (*a*) cambium stimulation with callus formation (*b*) root formation (*c*) bud inhibition, (*d*) an action on leaf growth (*e*) isodiametric growth of cells causing general swelling The last is typical of high auxin concentrations in many plants (see below)

2 *Cambium*

A clear cut promotion of cell division is produced in the cambium of many plants by treatment with auxin This was first demonstrated by

Snow (281), who had previously shown that some diffusible substance causes activation of the cambium in grafting, and indeed had been foreshadowed by Jost forty years earlier. The amount of indoleacetic acid necessary to cause cell divisions in the cambium of sunflower hypocotyls was shown to be comparable with the amount normally produced by buds and young leaves, as determined by Thimann and Skoog (331) in diffusion experiments. Thus cambial activation by auxin is a normal plant process (173a,281,292,293a). The activation which travels from the opening buds downward throughout the stem in the springtime is hence due in the main to auxin. Vigorous cambial activation, *i.e.*, cell division, was shown to result from auxin treatment of twigs of willow and poplar by Söding (291), who also showed (293) that the auxin travels polarly from apex to base in the twigs, mainly in the cambium itself. Before the buds open, organic matter migrates to them in considerable amount (55) and auxin begins to be liberated thereafter, as actual opening proceeds. That this auxin moves downward in a wave lasting only a few weeks was made clear by Zimmerman (377); this movement is followed closely by division of the cambium. The close time relations were shown clearly in apple by Avery *et al.* (16), who compared sections of the wood at different times in the spring, and different distances from the bud, with *Avena* test determinations of the auxin coming from the buds. It is characteristic of experiments with applied auxin that the cambial activation is generally limited to a few centimeters below the point of application, while the natural stimulus moves to ground level or even into the roots (292), it is important, therefore, that if the application is made within a limited period in the spring the resulting cambial activation can also travel great distances (105). Apparently, however, the active substance in cambium is not auxin alone, for Söding (293a) finds that cambium-stimulating preparations obtained from cambium itself are more active than the corresponding concentration of indoleacetic acid (see Chap. III, Section 1). Söding found that more auxin diffuses from the cambium than from any other tissue, in woody stems, and this has been confirmed for a number of tropical plants by Kramer and Silberschmidt (173a). There is, of course, no reason to believe that the auxin *is* responsible for differentiation into xylem and phloem.

When trees grow in a leaning position the wood formed on the underside is reddish and of characteristic morphology; this was described in 1896 by R. Hartig and such wood termed "rotholz" or "redwood." Wershing and Bailey (362) were able to duplicate this in white pine seedlings by auxin application and it is likely, therefore, that the extra auxin accumulated geotropically on the lower side of the stem is responsible for the natural phenomenon. If this is true, the great excess of

auxin applied in Soding's experiments (291) should also have produced redwood, a point which deserves further anatomical study

Application of auxin to woody twigs or cuttings also causes the formation of so-called callus, particularly at the basal cut surface (see 87, 182, and many others) The weight of callus so formed on poplar varies directly with the concentration of auxin applied (254), but again it falls off rapidly with increasing distance and reaches zero at about 3 cm below the point of application

3 *Other Tissues*

In Snow s experiments (281) only the cambium divided as a result of application of auxin at physiological concentrations, but later Kraus, Brown, and Hamner (175) and Hamner and Kraus (129) found the endodermis very reactive when the auxin concentrations were higher In young bean stems, mature vacuolated cells of many tissues enlarged and divided, later forming many root initials Tomatoes (37) and four o clock (*Mirabilis*) (128) behaved similarly It should also be pointed out that formation of root initials always involves very active cell division which often originates in the pericycle, but may occur in every living tissue from epidermis to pith (see Section VI above)

The first result of application of high auxin concentrations to young stems or hypocotyls is a very great swelling of the pith and cortical parenchyma (28 75 175,182) The same thing happens at the base of auxin treated cuttings (71,301, and casual observation of many workers on root formation) In these swellings starch is rapidly hydrolyzed (21,203,208), then organic materials are transported to the swelling from adjoining parts of the stem (206,207,301), the cells, particularly of the cortical parenchyma, increase greatly in size, while those of the epidermis shorten (see especially Figs 32 and 36 of Diehl *et al*, 75) Very large cells are also formed in tissue cultures exposed to auxin concentrations above 1 mg /l (98, pp 97–100) Cell division comes relatively late, usually after several days, and is seen in many tissues It is of interest that the nuclei in such swellings reveal chromosome doubling, tetraploid and even octoploid cells are formed (74) Similar polyploidy occurs in the callus tissue growing on cut surfaces of the stem after auxin treatment (109), although it is not clear how far this is due to the auxin, since polyploid tissue occurs also in natural calluses The auxin induced swellings of roots contain nuclei with internal chromosome multiplication also (192)

4 *Pathological Changes*

Galls on stems, and nodules on the roots of legumes, both involve numerous and continued cell divisions In the case of galls due to the

crown gall bacterium (*Phytomonas tumefaciens*), Link and Eggers (196) have shown that the infected tissues are very rich in auxin, and Brown and Gardner (51) and Link *et al.* (198) have produced gall-like growths by continued application of indoleacetic acid to the cut surface of a young bean plant after decapitation. Naphthaleneacetic acid and its amide can also produce gall-like swellings (176,203). However, in later stages of the growth of crown galls, neither auxin (252) nor even the bacteria (364) can be identified, so that an explanation based on auxin production by the bacteria cannot account for all the phenomena of crown gall. Indeed, secondary galls were produced by sterile inocula from the original galls by White and Braun (361), which indicates that the host cells have been permanently altered, as in animal cancer. This phenomenon was shown more strikingly by *in vitro* grafts of tumor tissue to sections of normal stems (255). In this work de Ropp shows (as the Wisconsin workers had done earlier) that crown galls on the intact plant in many respects behave as though they produce auxin, since they cause root formation, root thickening, and sometimes bud inhibition in adjacent normal tissues. However, the comparison is not perfect because in the grafts the only effect on the normal tissue is that of disorganized proliferation and roots are not formed, while in normal tissue proliferation occurs only at very high indoleacetic acid concentration and at all physiological levels roots are formed. He concludes that the diffusible "tumefacient factor" is probably not identical with auxin.

Nodules on legume roots are also very rich in auxin (194,195,313,315); unlike most auxin in plant tissues this material is wholly free and rapidly extractable (332). Since the invading rhizobia certainly form auxin in culture media (59,313), Thimann (313) proposed the following picture for nodule formation: the invading bacteria form considerable amounts of auxin, which causes cell division in the endodermis or pericycle. Such division would normally lead to the formation of a secondary root, but since the elongation of roots is strongly inhibited by auxin (see Section VII) the result is a more or less isodiametric swelling. Kraus (174), however, states that in nodule formation the first cell divisions occur in the cortex, so that the nodule is not strictly homologous with a lateral root.

B. Formation of Fruits

As long ago as 1909 Fitting found that the swelling of the ovary of certain orchids, which normally follows pollenation, can be brought about by applying extracts of the pollinia. Morita (210) later obtained similar results, and Laibach (179) showed that the active substance, both of orchid and of *Hibiscus* pollen, could be extracted with ether. Further,

the extract behaves like auxin and its effect can be duplicated with ether extracts of urine, etc (180) Pollen of many plants contains an auxin active on *Avena* (112,309,335) Yasuda (368), using aqueous extracts of pollen, obtained quite large swellings of the ovaries of *Solanum* and also (369) almost normal-looking fruits of cucumber Since these were formed without fertilization they were seedless or "parthenocarpic"

Final proof that this reaction is due to auxin was given by Gustafson (111), who produced mature seedless fruits of tomato and other plants by applying indoleacetic acid and other auxins in lanolin paste, to the styles before fertilization could occur Mature seedless pepper, crookneck squash, and even watermelon were produced by Wong (366), holly and strawberries by Gardner and Marth (90), pears by Sereiski (268), and other fruits in the same way For commercial use a mixture of seedless and fertilized fruits with a total increase in the number of fruits set is often sufficient

The method of application has been the subject of considerable practical study Gardner and Marth (90) used a water spray Howlett (142,143) a lanolin water emulsion, and Strong (300) a mixture of auxin with trigamine or morpholine applied to the entire flower bud cut off just above the ovary Zimmerman and Hitchcock (372 373) obtained seedless fruits of holly by means of the vapors of auxin esters, and of tomatoes with an aerosol of auxin esters (373) Both these treatments were applied to the whole plant To obtain completely seedless fruits of course, the styles must be removed before the pollen tubes can have grown through, but Howlett (142,143) has shown that, at least in the tomato, pollenation is often imperfect, so that for practical growers purposes the flowers can be left intact and, after spraying, the growth of all fruits is promoted by the auxin treatment Blossom end rot and bud inhibition often occur in sprayed fruit A list of parthenocarpic fruits produced by auxin up to 1942, and also a list of the plants which produce them naturally, is given in the review of Gustafson (119)

The relative activity of different auxins for parthenocarpy, though not easy to determine accurately, seems to place the different substances about in the same order as for root formation, or perhaps for primary growth promotion (see Section III, C), but not in the same order as in the *Avena* test or the pea test Gustafson (113 115,121) found α naphthoxyacetic and indolebutyric acids the most active, but later the di and tri chlorophenoxyacetic acids were found to be much more active (372,373) Such relative activities are doubtless determined, at least in part, by relative stability to plant enzymes under the long exposure involved in this type of experiment Should the finding of Tang and Bonner, *i e*, that the inactivating enzyme system in the pea is specific for indoleacetic

acid, be extended to the tomato and other plants, it would provide a good explanation for the relatively low activity of indoleacetic acid for parthenocarpy

The mechanism of this phenomenon is not fully understood, but a tentative picture has been presented by Gustafson (114,120) The auxin introduced either by the pollen or by artificial application starts growth by cell enlargement in the ovary tissues This, in fertilized fruit, leads to growth of the ovules themselves, and they then secrete auxin (their natural auxin content is high) in sufficient amount to cause continued growth of the ovary tissues Plants which readily produce parthenocarpic fruit, such as the navel orange, contain somewhat more natural auxin in the ovary walls than other varieties of the same species which do not show parthenocarpy It is this auxin in the ovary walls which then suffices for further growth after the first "shot" of auxin has initiated it This concept is based on auxin determinations in various parts of fruits of different species and varieties, and their correlation with parthenocarpy or even (120) general fruitfulness, the data are, however, not wholly clear-cut and the picture may need extensive modification In particular the concept that auxin secretion does not begin until growth has been started needs clarification There are certain suggestive parallels here with the growth of buds, in which the initial stimulus is furnished not by auxin (which inhibits) but by other factors, but thereafter auxin production follows growth

C Role of Auxin in Seed Germination

It was first shown by Cholodny (62) that oat seeds treated with auxin show a subsequent stimulation of growth This he compared to the effects of vernalization in which the seeds are moistened and then kept cool for a long time, under such conditions auxin is set free within the endosperm in considerable quantities, by enzymic action (61,271,342) The nature of the precursor in the endosperm, which liberates the auxin, is discussed in Section III, and need not concern us here The auxin set free in the endosperm does not, as it now appears, operate to produce vernalization, for Gregory and Purvis (110,245) have shown that the isolated embryo, freed from endosperm, can show normal vernalization, while Hatcher (130) finds no auxin in the rye embryo during germination at normal or vernalization temperature The acceleration of growth following treatment of the seed with auxin is a purely vegetative phenomenon Using indoleacetic acid, Thimann and Lane (324) showed that the inhibition of root growth which first appears after auxin treatment is later followed by an acceleration both of elongation and of branching, *i e*, formation of secondary roots and they ascribed the improved top

growth to this effect, which would lead to an increased total root system, indeed the roots of full grown oat plants so treated showed a large increase in weight over controls Amlong and Naundorf (9) obtained similar growth accelerations with many seeds, including sugar beets, which gave an increased yield of sugar per acre as a result It is important that the stimulation of growth although it may not be very large, lasts throughout the life of the plant, at least in some cases However, several other workers (*e g*, Barton, 20, Templeman and Marmoy, 307) have failed to obtain any appreciable effect from seed treatments so that the conditions of treatment are apparently quite critical and need further analysis Podesva (241a) reports good results with several vegetables

IX Mechanism of the Action

It will be clear from the preceding sections that the effects of auxin on plant cells are numerous Growth by increase in size is the major and most direct effect, but stimulation of cell division, without increase in size, in the cambium, in root initials, and in fruit formation is at least as important Clear-cut inhibitions of growth of buds roots, and the abscission layer appear also to be direct effects The action of auxin on the cell must therefore be a fundamental one, a kind of 'master reaction" The consequences of the process may lead to growth inhibition, etc, according to the supply of other factors and to the age and morphology of the tissues concerned

A Effects on Cell Wall

Before* it was recognized that phenomena other than simple cell enlargement were involved, Heyn (132,133) and Soding (289,290) brought forward considerable evidence that the effect of auxin, at least in the coleoptile, was to increase the plasticity of the cell wall The plant cell differs, of course, from that of the animal in its relatively rigid cellulosic wall, which resists the osmotic tendency of external water to enter and thus holds the cell size in balance Increased plasticity would decrease the pressure of the wall on the cell contents and thus allow water to enter osmotically, increasing the cell size The evidence was obtained by applying known loads to the plasmolyzed coleoptile or other organs, and measuring the irreversible or plastic stretching which resulted (135) Another method is to plasmolyze the plants after they have produced a curvature in response to auxin, the decrease in curvature resulting is in the part which was purely elastic

* A full discussion of the early work up to 1937 is given in Chapter VIII of *Phytohormones* (360), and by Heyn (135)

The plasticity of the coleoptile was found to decrease following decapitation and to increase again with the "regeneration of the physiological tip" after about 2 5 hours Application of auxin in agar to coleoptiles, flowerstalks, or stems clearly increased the plasticity Some of these experiments have been more recently repeated by Burkholder (53) with similar results Also auxin in lanolin gave essentially the same effects (256) It is clear that it is the change in plasticity, not in elasticity, which parallels change in growth rate This is particularly striking in roots, where auxin acts to increase the elasticity, whether it causes increase or decrease of the growth rate (51) The conception of growth which is involved is that the wall, after being made more plastic, is stretched by the entering water and then fixed in its stretched state by the interposition or apposition of new cellulose particles Bonner's measurements (32) of the weight of the cell walls indicate that, when growth occurs at 2°C the latter process lags behind, when it occurs at 23°, or in the presence of sugar, cell wall deposition exceeds growth and the weight per unit length increases However, it seems that some minimal cell wall deposition must keep pace with extension

A modification of the above view, according to which the auxin acts mainly on the pectic substances of the middle lamella, has been put forward by Ruge (256–258), with, however, insufficient experimental support According to his data this pectic material, which is said also to contain hexosans and hexonic acids, swells in auxin and it is this swelling which leads to growth To a lesser degree the swelling is also caused by acid pH which is known to promote growth (31,311) Hydrolytic enzymes are also claimed to promote growth through hydrolysis of the pectin although it has been known since the work of Seubert (269) that commercial enzyme preparations commonly contain some auxin

A more extensive consideration of the effect of auxin on cell walls, based both on experiment and on theory, has been set forth by Diehl *et al* (75) These workers believe the action is first exerted on the inter micellar substance which is probably of the nature of a wax (367), and thereafter on the cellulose micelles themselves The skeleton of the primary wall according to the observations and concepts of Frey-Wyssling (88) consists of micelles of cellulose oriented (statistically) perpendicular to the axis of elongation This skeleton has to be continuously modified to allow growth Unpublished observations of the author and T Kerr indicate that this takes place by a continual loosening and re-forming of the linkages between crisscrossed micelles, with simultaneous deposition of new micelles of the same orientation, these, although statistically perpendicular to the longitudinal axis actually lie in a double spiral at a moderate angle on either side of that axis However, these

conclusions are still uncertain, and a detailed discussion of the relation between growth and wall structure here would take us too far afield

There can scarcely be any direct chemical relation between wall deposition in growth and the auxin which causes it, because the measurements and calculations of Thimann and Bonner (319) show that each auxin molecule causes the deposition of some 3×10^5 hexose residues as cellulose, as well as the pectin, hemicellulose, and protein, which also are laid down Further, the amount of wall formed per molecule of auxin varies with temperature

With the recognition of the other effects of auxin, the field widened Two main viewpoints have focussed much of the research

B Mobilization of Special Hormones

In brief, this view is that each process, except cell enlargement, is brought about by a specific hormone, there would be a root forming substance, a stem forming substance, a bud inhibiting substance etc These substances are discussed in more detail in Section V of the following chapter, it is only necessary here to consider their relation to auxin The action of auxin is visualized as causing the mobilization of these substances at the point at which the auxin accumulates As an example, rooting of a cutting would be due to (*1*) the polar transport of auxin to the base and its accumulation there, (or its direct application at the base), (*2*) the consequent accumulation of the root-forming hormone, "rhizocaline" at the base, and (*3*) action of the latter substance on the basal tissues Similarly, swelling of the stem at the point of auxin application would be due to the mobilization by the auxin of "caulocaline" and other substances necessary for stem growth This view has been put forward especially by Went (359, see Sect V of Chapter III) but other authors, notably Gautheret (96), have explained their results in terms of numerous specific hormones

Pending definite proof of the existence of such special hormones, this concept is difficult to prove or disprove Growing loci in the plant certainly manage to accumulate water, carbohydrates, and other materials for growth, for instance in the formation of swellings The data of Stuart (301) and Mitchell and Stewart (206), showing a marked increase of dry weight in the region where auxin is applied to a stem, are particularly clear in this connection There is enough movement of materials to cause strong inhibition of growth above the point of application (204,284) Thus in an indirect way it must be true that auxin leads to the "mobilization' of such substances The difficulty comes when the effect of auxin on isolated plant parts is considered Thus, sections of coleoptile 3 mm long immersed in solutions of auxin and sucrose, will

grow some 100% (262) Fragments of *Helianthus* hypocotyl (255), or of potato tuber (123) will form roots vigorously in response to auxin Isolated buds in solution are inhibited by auxin (272), so are isolated root tips (86, see Section VII, B) In all these instances it is difficult to ascribe any role to mobilization, yet the effect of auxin is very similar to that in the intact plant If, however, we conclude that the evidence for the mobilization of specific hormones is insufficient, at any rate at the present time, then the alternative is that the varied effects of auxin are due to differences in the ability of different tissues to respond (314) This brings us back to the starting point and calls for a closer study of the intimate nature of the action of auxin in the cell

C Relation between Respiration and Growth

It has been known for a long time that growth of the coleoptile will not take place anaerobically, and Bonner in 1933 showed that growth is inhibited by cyanide, and to the same extent for a given concentration as is respiration However, neither Bonner (33) nor van Hulssen (146) could find any acceleration of the respiration of the coleoptile by auxin alone Hence it was concluded only that respiration is "a formal prerequisite for growth" and not that any respiratory process is involved in growth Later work, however, has shown that the relationship is closer than that

In the first place, cyanide is not the only inhibitor of respiration which also inhibits growth Commoner and Thimann in 1941 found that iodoacetate is still more effective A concentration of $2\ 10^{-5}$ *M*, after a few hours delay, inhibits growth completely This concentration, however, has little effect on respiration of the coleoptile, which requires about ten times as high a concentration for marked inhibition (Fig 9) Since iodoacetate inhibits numerous dehydrogenases, they deduced that there is a special dehydrogenase system which takes part somehow in growth, though it cannot be responsible for more than a very small part of the respiration Recently Bonner and Wildman (35) have made a similar discovery with respect to fluoride, namely, that low concentrations inhibit growth but do not appreciably reduce the oxygen consumption of the coleoptile Iodoacetate and fluoride, of course, are both active on stages of the phosphorolysis cycle, and Thimann and Bonner have reported (320) that glucose-1-phosphate releases the inhibition by fluoride From the work of James, James, and Bunting (147) it appears that the phosphorolysis cycle in plant tissue, at least in barley leaves, is similar to that in yeast or muscle, being inhibited by fluoride or iodoacetate On the other hand, Commoner and Thimann found the iodoacetate inhibition to be reversed by malate, succinate, fumarate, and pyruvate, and

concluded that the four-carbon acid oxidation system was the one involved This is supported by the finding of Albaum and co-workers (2,3) that intact oat seedlings are also inhibited in growth by iodoacetate and the inhibition reversed by the four carbon acids However, Albaum and Eichel (3) find that with intact seedlings the iodoacetate inhibition is also reversed by malonic and maleic acids, which should (in animal tissues and bacteria at least) inhibit the four-carbon acid system Since also Berger and Avery (25) were unable to find any evidence for succinic dehydrogenase in the coleoptile, it must be concluded that at present the exact nature of the enzyme system involved in growth is not established

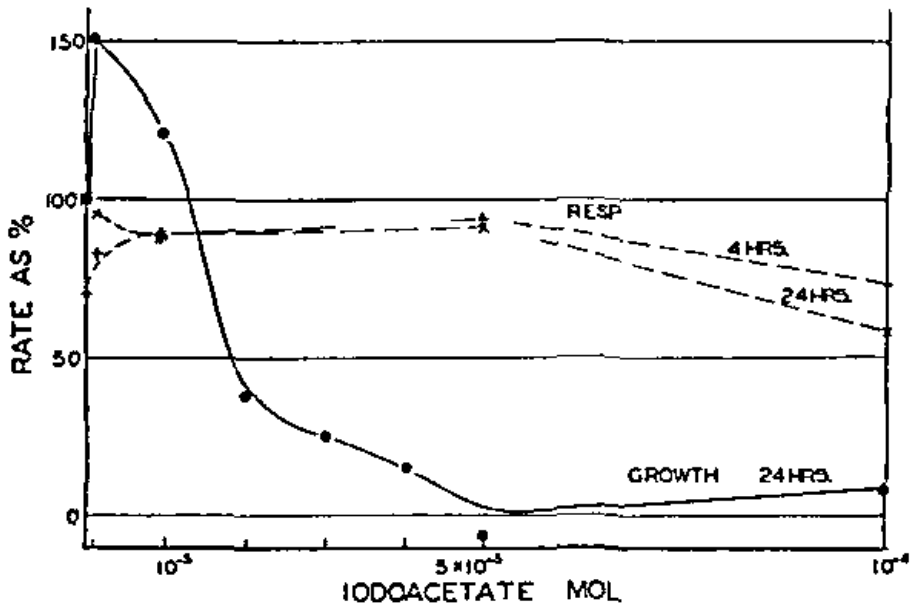


FIG 9 —The effect of iodoacetate on the growth (solid line) and respiration (dashed line) of *Avena* coleoptile sections Growth may be very largely inhibited with little decrease in respiration (From Commoner and Thimann, 70)

One of the key enzymes is doubtless of sulfhydryl nature and its concentration appears to decrease with increasing age of the coleoptile (335a)

Very remarkable support for the conceptions of Commoner and Thimann comes from the work of Ryan, Tatum, and Giese (259) on an entirely different growth system, that of the fungus *Neurospora* Here also iodoacetate inhibits growth while respiration is less sensitive, at about $3\ 10^{-3}$ *M*, growth is reduced to zero while 30% of the respiration remains Provided the iodoacetate concentration is not too high, the inhibition is released by succinate, fumarate, or malate, and to a lesser extent by pyruvate The relation between growth and respiration in *Neurospora* is somewhat closer than in *Avena*, and Ryan *et al* point out that inhibition of growth parallels that of respiration under certain condi-

tions if only the iodoacetate-sensitive part of respiration is considered Such a close parallelism does not exist in *Avena*

Not only is respiration linked to growth, but it is also directly affected by auxin Commoner and Thimann confirmed the older observations (see above) that coleoptile sections in water show no increased oxygen *consumption when* indoleacetic acid is added, but found that if the sections have been kept a few hours in sucrose there is a definite rise in respiration immediately on addition of indoleacetic acid (1–10 mg /l) After some hours in malate the rise is larger, 20-35% The former fact but not the increased effect of malate was confirmed by Berger *et al* (26)

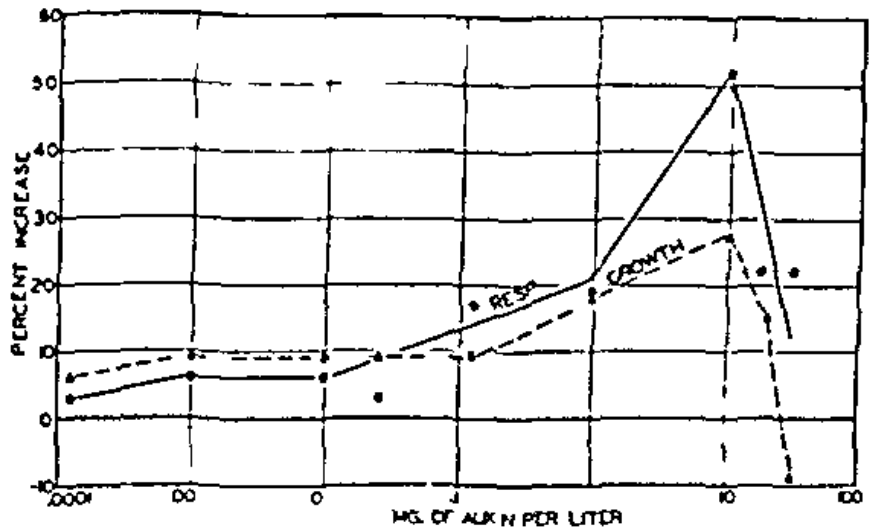


Fig 10 —The parallelism between the effects of auxin on growth and on respiration of coleoptile sections which have previously been soaked 18 hours in sucrose (1%) plus malate (0 001 *M*) (From Commoner and Thimann 70)

who found indeed still larger increases due to indoleacetic acid in presence of sugar The effect of different auxin concentrations on respiration in presence of malate shows a very close parallel to their effects on growth (Fig 10) There can be little doubt, therefore, that the growth process involves a respiratory enzymic reaction as an integral part, and that auxin in some way accelerates or acts as a coenzyme for this reaction

D Relation between Growth and Protoplasmic Streaming

In his fundamental experiments on auxin, Went (348) noted the speed of protoplasmic streaming in the coleoptile and suggested that it might be responsible for auxin transport While this has been neither confirmed nor disproved it has become increasingly probable that streaming is connected with the growth process and the effect of auxin In studying the effect of light on growth Bottelier (38 39) discovered some remarkable

parallels between streaming and growth Exposure to light temporarily retards the rate of streaming as also the rate of growth, and the proportion between the effectiveness of different wavelengths is the same for streaming as for growth Further, both streaming and growth show a similar dependence upon oxygen, which varies with age of the coleoptile This was shown indirectly by following the effect of temperature on streaming rate (39) The rate increases with temperature according to the usual van't Hoff relationship but flattens off at about 21° in young (96-hour) coleoptiles, this flattening can be prevented by saturating the water with oxygen Old (260-hour) coleoptiles show no such flattening of the curve, which continues upward to 33° Even in old coleoptiles the curve can, however, be flattened by bubbling nitrogen through the water The rate at which oxygen is consumed for streaming therefore decreases with increasing coleoptile age

This fact was confirmed by Thimann and Sweeney, who subsequently made an extensive study of the effect of auxin on protoplasmic streaming in the coleoptile They first found (334) that auxin in physiological concentrations produces a temporary acceleration of the streaming rate, which returns to normal after about twenty minutes If, however, sugar is added, the acceleration is maintained for several hours (304), as is the growth rate (see Fig 11 A) The acceleration is dependent on the access to oxygen, it cannot be obtained after infiltration of the intercellular spaces with water (224,302), nor during treatment with dinitrophenol (334), which presumably increases the rate of oxygen consumption and thus lowers the oxygen tension in the solution Further analysis (305) showed that, when the conditions are such that auxin alone will not accelerate the streaming, simultaneous treatment with auxin and malate produced a *maximal acceleration These conditions include (a)* very dilute auxin (indoleacetic acid 0 001 mg /l), (*b*) coleoptiles too old (6 days old), and (*c*) coleoptiles cut off and soaked 24 hours in water or fructose solution (Fig 11 B) Finally, the acceleration is prevented by iodoacetate in the same concentration as it prevents growth, namely, 5×10^{-5} *M* , and this inhibition is reversed by malate The data thus indicate that the basal streaming rate is not influenced by auxin, auxin however, accelerates the rate through influencing an oxidative reaction involving sugar and malate, which is most probably the same reaction as that which leads to growth It is interesting to note that in old coleoptiles, in which elongation cannot occur because secondary wall has been laid down, the typical acceleration of streaming by auxin and malate may still take place In other words, the fundamental (enzymic) growth process need not necessarily cause visible growth (see 305,317) Since the streaming acceleration occurs before any detectable growth accelera

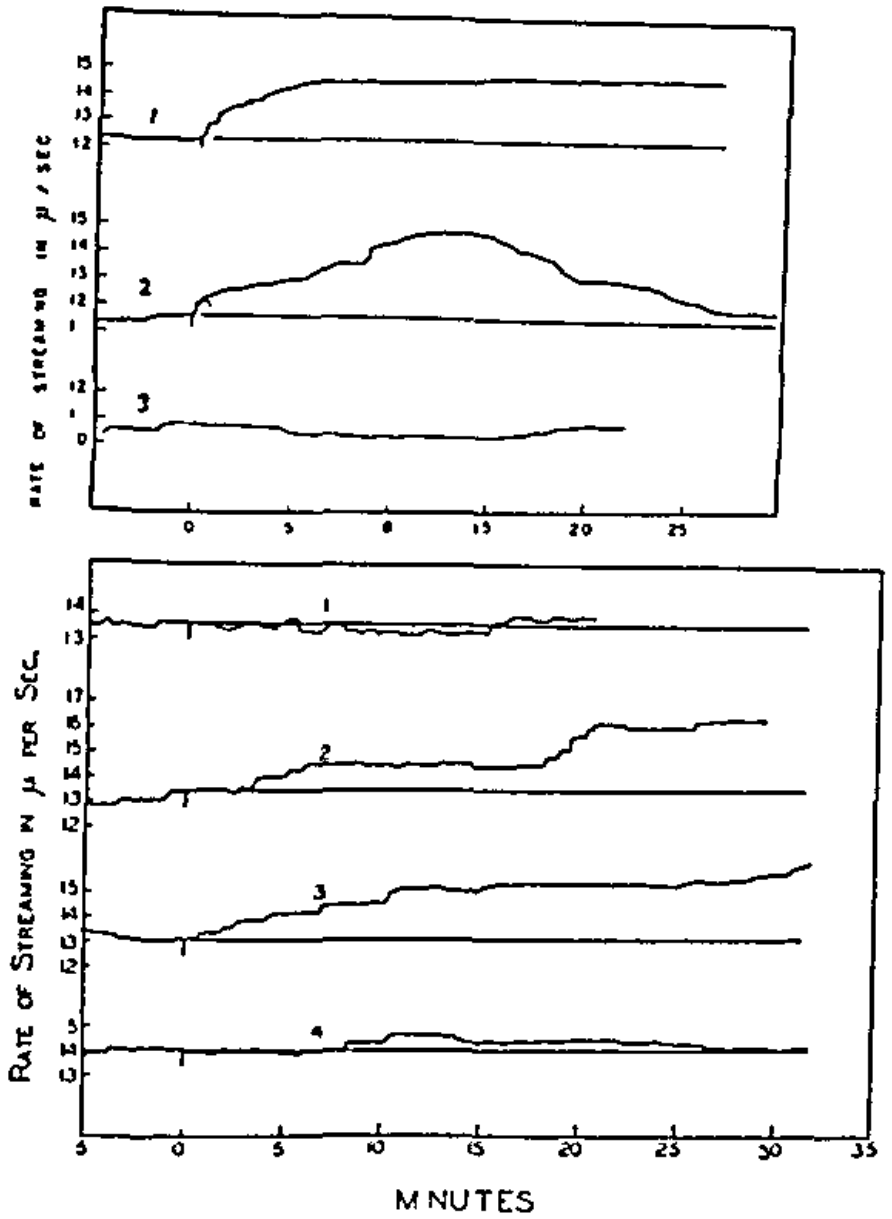


FIG 11.—Records of the rate of protoplasmic streaming in coleoptile epidermal cells in red light. Above: 1. Effect of auxin (1 mg. per liter) plus fructose (1%). 2. Effect of auxin (1 mg. per liter) alone. 3. Control in water. (From Sweeney, 302.) Below: 1. Soaked in fructose (1%), treated with auxin (1 mg. per liter). 3. Soaked in fructose (1%) plus malate (0.001 *M*), treated with auxin plus malate. 2. Soaked in water, treated with auxin plus malate. 4. Soaked in water, treated with malate alone. (From Sweeney and Thimann, 305.) Auxin (indoleacetic acid) added at time zero in each case.

tion, it may well be the *cause* of the accelerated growth. It is possible, too, that the acceleration of streaming is the means whereby accelerated accumulation of plastic materials for the growth process (see pp. 52, 57) is brought about.

As shown in Section VII, B, the growth of roots is inhibited by all

but excessively low concentrations of auxin It is of interest that Sweeney (303) finds that the rate of streaming in root hairs of *Avena* is accelerated by much lower auxin concentrations than in the coleoptile, the optimum concentration being 10^{-4} mg /l as against about 0 1 mg /l in the coleoptile cells Inhibition of streaming also takes place at somewhat lower concentrations than in the coleoptile, but curiously enough, removal of the seed and coleoptile seems to reduce the sensitivity of the root hairs to high auxin concentrations Sweeney also found that streaming continues at the normal rate in fully plasmolyzed root hairs, thus making it unlikely that streaming has its inception at the protoplasm–cell wall interface

The way in which the streaming rate could be affected by auxin is, of course, unknown Northen (222) has found that treatment with auxin decreases the viscosity of protoplasm, and that this effect parallels at least roughly, the effects on growth While a reduction in viscosity would doubtless lead to an increase in the rate of flow, the causal connection, if any, will need to be established by studying both phenomena on the same material Probably both are related to the respiratory effects described above

E Growth and Uptake of Water

In its simplest form, the enlargement of plant tissues can be considered as depending on uptake of water This must of course be accompanied or followed by synthesis of protoplasm and of cell wall Since isolated sections of stems or coleoptiles will, however, grow 100% or more in sugar and auxin alone, nitrogen uptake and protein synthesis evidently is not an integral part of the primary growth process The experiments of Reinders with slices of potato and other materials are therefore of considerable interest because, instead of measuring elongation, Reinders (250,251) measured increase in weight in water (or auxin solution), which is a direct measure of water uptake In general, her results are like those with coleoptile sections in that auxin (especially indoleacetic acid, 1 mg /l) strongly promotes water uptake in a strictly aerobic process Dry-weight losses indicate that the auxin also stimulates respiration in this material, particularly in the later stages of an experiment lasting several days If auxin exerts its effect directly in reducing the plasticity of the cell walls, as in the view of Heyn and Soding, then the increased water uptake would be accounted for at once on osmotic grounds This, however appears not to be the case Thimann and Schneider (326) showed that low concentrations of potassium chloride considerably promote growth in auxin solution, and that growth of coleoptile sections is a linear function

of the osmotic gradient. This last point was established by using mannitol, to which plant cells are highly impermeable (65,66), in the external solution; van Overbeek (cited in 326) has reported similar results with sucrose. Commoner, Fogel, and Muller (68) have shown that the water intake can occur against an osmotic gradient, *i e*, in presence of sucrose solution of plasmolyzing concentration. Conductivity measurements (Commoner and Mazia, 69, and unpublished data)[10] show that the potassium chloride, as well as the water, is taken into the tissue against the osmotic gradient Commoner *et al* also showed that this water uptake is inhibited by iodoacetate It is, however, true that growing tissues show no change in their osmotic pressure, as against nongrowing ones, particularly when in auxin without sugar (234), so that the water and electrolyte must be taken in strictly parallel with growth, and perhaps the osmotic pressure may equally be maintained internally by starch hydrolysis Indeed, auxin does promote starch hydrolysis (208) It is tempting to consider the salt uptake to be the primary process, for, as Commoner (67) points out, salt uptake is, like growth, well known to be typically an aerobic process, requiring carbohydrate and associated with active protoplasmic streaming (140,295,296) On the other hand, starch-rich tissues like potato grow to a considerable extent in distilled water, as shown by Reinders (251), so that uptake of externally applied salts is not necessarily a feature of primary (short-term) growth. Further analyses of these relationships will undoubtedly shed important light on the fundamental nature of growth

F Conclusions

The general concept of auxin action which emerges from the facts presented can be summarized as follows:

The auxin may produce a variety of different effects, depending on: (*a*) its concentration, (*b*) the tissues on which it acts, (*c*) its stability in these tissues, and (*d*) the relative ease with which it is transported in the plant These different effects in all probability spring from one fundamental master reaction in the cell

The structural requirements for auxin action point to the need for a particular set of polar groupings in a particular spatial array, *i e*., they suggest that the molecule has to combine with a determined spatial structure

There is abundant evidence that auxins combine with proteins, and though the exact nature of the combination is obscure, it is probable that

[10] The author desires to thank Dr Commoner for making available unpublished data and discussion

different types of combination may occur, and certain that many different proteins are involved

The auxins act catalytically

The action involves a respiratory process which concerns carbohydrate and the organic acids, this process is linked directly with the protoplasmic streaming

If we put these simplified conclusions together, it is evident that they point in one direction auxin is a coenzyme (or prosthetic group) for some fundamental enzymic process in the cell This process is a bottleneck, or limiting factor, through which the uptake of solutes and/or water, the deposition of cellulose, and all the other appurtenances of growth must flow Which process is the primary one, if any, and which are secondary remains unsolved

REFERENCES

1 Albaum, H G *Am J Botany* **25**, 124–133 (1938)
2 Albaum, H G , and Commoner, B *Biol Bull* **80**, 314–323 (1941)
3 Albaum, H G and Eichel, B *Am J Botany* **30**, 18–23 (1943)
4 Albaum, H G , Kaiser, S , and Nestler, H A *ibid* **24**, 513–515 (1937)
5 Algeus, S *Botan Notiser (Lund)*, 129–278 (1946)
6 Amlong, H U *Planta* **21**, 211–250 (1933)
7 Amlong, H U *Jahrb wiss Botan* **83**, 773–780 (1936)
8 Amlong, H U *ibid* **88**, 421 (1939)
9 Amlong, H U , and Naundorf, G *Forschungsdienst* **5**, 292–303 (1938)
10 Arisz, W H *Proc Konink. Akad wetenschappen Amsterdam* **45**, 2–8, 794–801 (1942), **53**, 236–260 (1944)
11 Asana, R D *Ann Botany* **2**, 955–957 (1938)
12 Avery, G S , Jr *Bull Torrey Botan Club* **62**, 313–330 (1935)
13 Avery, G S , Jr , Berger, J , and Shalucha, B *Am J Botany* **28**, 596–607 (1941)
14 Avery, G S , Jr , Berger, J , and Shalucha, B *Botan Gaz* **104**, 281–287 (1942)
15 Avery, G S , Jr , Berger, J , and White, R O *Am J Botany* **32**, 188–191 (1945)
16 Avery, G S , Jr , Burkholder, P R , and Creighton, H B *ibid* **24**, 51–58 (1937a)
17 Avery, G S , Jr , Burkholder, P R , and Creighton, H B *ibid* **24**, 553–557 (1937b)
18 Avery, G S , Jr , Creighton, H B , and Hock, C W *ibid* **26**, 360–365 (1939)
19 Bachmann, F , and Bergann, F *Planta* **10**, 744–755 (1930)
20 Barton, L V *Contrib Boyce Thompson Inst* **11**, 181–206, 229–240 (1940)
21 Beal, J M *Botan Gaz* **102**, 366–372 (1940)
22 Beal, J M *ibid* **105**, 471–474 (1944)
23 Berger, J , and Avery, G S , Jr *Am J Botany* **31**, 11–19 (1944a)
24 Berger, J , and Avery, G S , Jr *ibid* **31**, 199–203 (1944b)
25 Berger, J , and Avery, G S , Jr *ibid* **31**, 203–208 (1944c)
26 Berger, J , Smith, P , and Avery, G S , Jr *ibid* **33**, 601–604 (1946)
27 Blackman, G E *Nature* **155**, 500–501 (1945)
28 Blum, J L *Botan Gaz* **102**, 737–748 (1941)
29 Bonner, D M *ibid* **100**, 200–214 (1938)

30 Bonner, J. *J Gen Physiol* **17**, 63-76 (1933)
31 Bonner, J *Protoplasma* **21**, 400–423 (1934a)
32 Bonner, J *Proc Natl Acad Sci U S* **20**, 393-397 (1934b)
33 Bonner, J *J Gen Physiol* **20**, 1–11 (1936)
34 Bonner, J , and Koepfli, J B *Am J Botany* **26**, 551–566 (1939)
35 Bonner, J , and Wildman, S G *Growth* **10**, Symp of Society for Growth and Development, 51-68 (1946)
36 Borgström, G The transverse reactions of plants Diss , Lund (1939)
37 Borthwick, H A , Hamner, K C , and Parker, M W *Botan Gaz* **98**, 491–519 (1937)
38 Bottelier, H P *Rec trav botan Néerland* **31**, 474–582 (1934)
39 Bottelier, H P *ibid* **32**, 287–292 (1935)
40 Bouillenne, R , and Went, F W *Ann Jard Bot Buitenzorg* **43**, 25–202 (1933)
41 Boysen Jensen, P *Ber deut botan Ges* **28**, 118–120 (1910)
42 Boysen-Jensen, P *ibid* **31**, 550–566 (1913)
43 Boysen-Jensen, P *Planta* **5**, 464–477 (1928).
44 Boysen-Jensen, P *ibid* **19**, 345–350 (1933), **20**, 688–698 (1933)
45 Boysen-Jensen, P *Kgl Danske Videnskab Selskab Biol Medd* **13**, 1–36 (1936a)
46 Boysen-Jensen, P Growth hormones in plants Translated and rev by G S Avery, Jr , and P R Burkholder, McGraw-Hill, New York, 1936b
47 Brauner, L *Kolloidchem Beihefte* **23**, 143–152 (1926)
48 Brauner, L *Jahrb wiss Botan* **66**, 381–428 (1927), **68**, 711–770 (1928)
49 Brauner, L , and Bünning, L *Ber deut botan Ges* **48**, 470–476 (1930)
50 Brecht, F *Jahrb wiss Botan* **82**, 581–612 (1936)
51 Brown, N A , and Gardner, F E *Phytopathology* **26**, 708–713 (1936)
52 Bünning, E *Planta* **26**, 719–736 (1937), **27**, 148–158 (1937)
53 Burkholder, P R *Am J Botany* **28**, 911–920 (1941)
54 Burström, H *Ann Agr Coll Sweden* **10**, 209–240 (1942), **13**, 1–86 (1945)
55 Burström, H , and Krogh, A *Kgl Danske Videnskab Selskab Biol Medd* **20**, 1–27 (1946)
55a DuBuy, H G *Rec trav botan Néerland* **30**, 793–925 (1933)
56 DuBuy, H G , and Nuernbergk, E *Ergeb Biol* **9**, 358–544 (1932), **10**, 207–322 (1934), **12**, 325–543 (1935)
57 Castan, R *Compt rend* **216**, 576 (1943)
58 Castle, E S *J Gen Physiol* **13**, 421–435 (1930), *Cold Spring Harbor Symposia Quant Biol* **3**, 224–229 (1935)
59 Chen, H K *Nature* **142**, 753–754 (1938)
60 Cholodny, N G *Jahrb wiss Botan* **65**, 447–459 (1926)
61 Cholodny, N G *Planta* **23**, 280–312 (1935)
62 Cholodny, N G *Compt rend acad sci U R S S* **3**, 8–9 (1936)
63 Clark, W G *Plant Physiol* **12**, 409–440 (1937)
64 Clark, W G *ibid* **13**, 529–552 (1938)
65 Collander, R *Trans Faraday Soc* **33**, 985–990 (1937)
66 Collander, R , and Bärlund, H *Acta Bot Fennicae*, **11**, 1–114 (1933)
67 Commoner, B In press (1947)
68 Commoner, B , Fogel, S , and Muller, W H *Am J Botany* **30**, 23–28 (1943)
69 Commoner, B , and Mazia, D *Plant Physiol* **17**, 682–685 (1942)
70 Commoner, B , and Thimann, K V *J Gen Physiol* **24**, 279–296 (1941)
71 Cooper, W C *Botan Gaz* **99**, 599–614 (1938)
72 Darwin, C , and F The power of movement in plants John Murray, London, 1880, New York, Appleton, 1888

73 Delisle, A L *Am J Botan* **24**, 159–167 (1937)
74 Dermen, H *J Heredity* **32**, 133–138 (1941)
75 Diehl, J M, Gorter, C J, van Iterson, Jr, G, and Kleinhoonte, A *Rec trav botan Néerland* **36**, 711–798 (1939)
76 Dijkman, M J *ibid* **31**, 391–450 (1934)
77 Doak, B W *New Zealand J Sci Tech* **21**, 336A–343A (1940)
78 Dolk, H Geotropie en groeistof Diss, Utrecht, 1930 Engl translation in *Rec trav botan Néerland* **33**, 509–585 (1936)
79 Dorn, H *Planta* **28**, 20–42 (1938)
80 Dostál, R *Acta Soc Sci Nat Moravicae* **3**, 83–209 (1926)
81 Dostál, R *Ber deut botan Ges* **54**, 418–429 (1936)
81a Dostál, R *Planta* **33**, 558–575 (1943)
82 Eigsti, O J *Proc Natl Acad Sci U S* **24**, 56–63 (1938)
83 Evenari, M, and Konis, E *Palestine J Botany Jerusalem Series* **1**, 13–26, 113–118 (1938)
84 Faber, E R *Jahrb wiss Botan* **83**, 439–469 (1936)
85 Ferman, J H G *Rec trav botan Néerland* **35**, 177–287 (1938)
86 Fiedler, H *Z Botan* **30**, 385–436 (1936)
87 Fischnich, O *Ber deut botan Ges* **56**, 144–152 (1938)
88 Frey-Wyssling, A *Protoplasma* **25**, 261–300 (1936) *Science Progress* **134**, 249–262 (1939)
88a Funke, H *Jahrb wiss Botan* **91**, 54–82 (1943)
89 Gardner, F E, and Cooper, W C *Botan Gaz* **105**, 80–89 (1943)
90 Gardner, F E and Marth, P C *ibid* **99**, 184–195 (1937)
91 Gardner, F E, and Marth, P C *Proc Am Soc Hort Sci* **37**, 415–428 (1939)
92 Gast, A *Ber schweiz botan Ges* **52**, 441–475 (1942)
93 Gautheret, R -J *Compt rend soc biol* **127**, 259–261 609–612 (1938)
94 Gautheret, R -J *Compt rend* **208**, 118–121 (1939)
95 Gautheret, R -J *Compt rend soc biol* **136**, 458–459 (1942)
96 Gautheret, R -J *Rev cyt cytophysiol vég* **6**, 85–180 (1943)
97 Gautheret, R -J *Compt rend soc biol* **138**, 395–396 (1944)
98 Gautheret, R -J La culture des tissus Gallimard et Cie, Paris, (1945)
99 Geiger Huber, M, and Burlet, E *Jahrb wiss Botan* **84**, 233–253 (1936)
100 Geiger Huber, M, and Huber, H *Experientia* **1**, 1–5 (1945)
101 Goodwin, R H *Am J Botan* **24**, 43–51 (1937)
102 Gordon, S A *ibid* **43**, 160–169 (1946)
103 Gordon, S A, and Wildman, S G *J Biol Chem* **147**, 389–398 (1943)
104 Gorter, C J, and Funke, G L *Planta* **26**, 532 545 (1937)
105 Gouwentak, C A, and Maas, A L *Mededeel Landbouw-hoogeschool Wageningen* **44**, 3–16 (1940)
106 Grace, N H *Can J Research C*, **15**, 538–546 (1937)
107 Grace, N H, and Thistle, M W *ibid C*, **18**, 122–128 (1940)
108 Granick, S, and Dunham, H W *Science* **87**, 47 (1938)
109 Greenleaf, W H *J Heredity* **29**, 451–464 (1938)
110 Gregory, F G, and Purvis, O N *Ann Botany NS* **2**, 237–251 (1938)
111 Gustafson, F G *Proc Natl Acad Sci U S* **22**, 628–636 (1936)
112 Gustafson, F G *Am J Botany* **24**, 102–107 (1937)
113 Gustafson, F G *ibid* **25**, 237–244 (1938)
114 Gustafson, F G *ibid* **26**, 135–138, 189–194 (1939)
115 Gustafson, F G *Botan Gaz* **102**, 280–286 (1940a)
116 Gustafson, F G *Science* **92**, 266–267 (1940b)
117 Gustafson, F G *Plant Physiol* **16**, 203–206 (1941a)

118 Gustafson, F G *Am J Botany* **28**, 947–951 (1941b)
119 Gustafson, F G *Botan Rev* **8**, 599–654 (1942a)
120 Gustafson, F G *Proc Natl Acad Sci U S* **28**, 131–133 (1942b)
121 Gustafson, F G *Proc Am Soc Hort Sci* **40**, 387–389 (1942c)
122 Gustafson, F G *Am J Botany* **30**, 649–654 (1943)
123 Guthrie, J D *Contrib Boyce Thompson Inst* **11**, 29–53 (1939)
124 Haagen Smit, A J, Dandliker, W B, Witwer, S H, and Murneek, A E, *Am J Botany* **33**, 118–120 (1946)
125 Haagen Smit, A J, Leech, W D and Bergren, W R *ibid* **29**, 500–506 (1942)
126 Haagen Smit A J and Went, F W *Proc Konink Akad Wetenschappen Amsterdam* **38**, 852 857 (1935)
127 Hamner, C L, and Tukey, H B *Botan Gaz* **106**, 232–245 (1944)
128 Hamner K C *ibid* **99**, 912–954 (1938)
129 Hamner, K C, and Kraus, E J *ibid* **98**, 735–807 (1937)
130 Hatcher, E S J *Ann Botan* N S **9**, 235–266 (1945)
131 Havránek, P Diss Tierarztl Hochsch Brunn, 1931 (cited by Dostál, 1936)
131a Hemberg, T *Svensk Botan Tid* **36**, 467–470 (1942), *Acta Horti Bergiani* **14**, 134–220 (1947)
132 Heyn, A N J *Rec trav botan Néerland* **28**, 113–244 (1931)
133 Heyn, A N J *Jahrb wiss Botan* **79**, 753–789 (1934)
134 Heyn, A N J *Proc Konink Akad Wetenschappen Amsterdam* **38**, 1074–1081 (1935)
135 Heyn, A N J *Bot Rev* **6**, 515–574 (1940)
136 Hitchcock, A E *Contrib Boyce Thompson Inst* **7**, 349–364 (1935)
137 Hitchcock, A E, and Zimmerman, P W *ibid* **7**, 447–476 (1935)
138 Hitchcock, A E, and Zimmerman, P W *ibid* **9**, 463–518 (1938)
139 Hitchcock, A E, and Zimmerman, P W *ibid* **11**, 143–160 (1940)
140 Hoagland, D R, and Broyer, T C *Plant Physiol* **11**, 471–507 (1936)
141 Hoffman, M B, Edgerton, L J, and Van Doren, A *Proc Am Soc Hort Sci* **40**, 35–38 (1940)
142 Howlett, F S *ibid* **39**, 217–227 (1941)
143 Howlett, F S *ibid* **41**, 277–281 (1942)
144 Hubert, B, and Beke, A *Mededeel Landbouw-hoogeschool Gent* **6**, 1–58 (1938)
145 Hubert, B, Rappaport, J Beke A, and Funke, G L *Mededeel Landbouw-hoogeschool Gent* **7**, 1–103 (1939)
146 Hulssen C J van Ademhaling Gisting en Groei Diss, Utrecht (1936)
147 James, W O, James, G M, and Bunting, A H *Biochem J* **35**, 588–594 (1941)
148 Johnston, E S *Smithsonian Misc Collections* **92**, 11–28 (1934)
149 Jost, L *Z Botan* **33**, 193–215 (1938)
150 Jost, L, and Reiss, E *ibid* **30**, 335–376 (1936)
151 Jost, L, and Reiss, E *ibid* **31**, 65–94 (1937)
152 Juel, I *Dansk Bot Arkiv* **12**, 1–16 (1946)
153 Koch, K *Planta* **22**, 1–33 (1934)
154 Kockemann, A *Ber deut botan Ges* **52**, 523–526 (1934)
155 Kockemann, A, *Beih Bot Centr* **55**, I abb 191–195 (1936)
156 Kodicek, E, Carpenter, K J, and Harris, L J *Lancet* **251**, 491–492 (1946)
157 Koepfli, J B, Thimann, K V, and Went, F W *J Biol Chem* **122**, 763–780 (1938)
158 Kögl, F *Ber* **68**, 16–28 (1935)
159 Kögl, F, and Erxleben, H *Z physiol Chem* **227**, 51–73 (1934) **235**, 181–200

160 Kögl, F Erxleben, H , and Haagen Smit, A J *ibid* **225**, 215–229 (1934)
161 Kögl, F , Erxleben, H , and Koningsberger, C *ibid* **280**, 135–147 (1944)
162 Kögl, F , and Haagen Smit, A J *Proc Konink Akad Wetenschappen Amsterdam* **34**, 1411–1416 (1931)
163 Kögl, F , Haagen Smit, A J , and Erxleben, H *Z physiol Chem* **214**, 241–261 (1933a)
164 Kögl, F , Haagen Smit A J , and Erxleben, H *ibid* **220**, 137–161 (1933b)
165 Kögl, F , Haagen Smit, A J , and Erxleben, H *ibid* **228**, 90–103 (1934a)
166 Kögl, F , Haagen Smit, A J , and Erxleben, H *ibid* **228**, 104–112 (1934b)
167 Kögl, F , Haagen Smit, A J , and van Hulssen, C J *ibid* **241**, 17–33 (1936)
168 Kögl, F , and Kostermans D G F R *ibid* **228**, 113–121 (1934)
169 Kögl, F , and Kostermans, D G F R *ibid* **235**, 201–216 (1935)
170 Kögl, F , and Schurings, G J *ibid* **280**, 148–161 (1944)
171 Kögl, F , and Verkaaik, B *ibid* **280**, 167 176 (1944a)
172 Kögl, F , and Verkaaik, B *ibid* **280**, 162–166 (1944b)
173 Koningsberger, C De auto-inactiveering der Auxinen Diss Utrecht (1936)
173a Kramer, M , and Silberschmidt, K *Arquiv inst biol Sao Paulo* **17**, 99–148 (1946)
174 Kraus, E J *Botan Gaz* **102**, 602–622 (1941)
175 Kraus, E J Brown N A , and Hamner K C *ibid* **98**, 370–420 (1936)
176 Kraus, E J , and Mitchell, J W *ibid* **101**, 204–225 (1939)
177 Kuhn, R , Jerchel, D , Moewus, F Möller L F and Lettré H *Naturwissenschaften* **31**, 468 (1943)
178 van der Laan, P A *Rec trav botan Néerland* **31**, 691–742 (1934)
179 Laibach, F *Ber deut botan Ges* **50**, 383–390 (1932)
180 Laibach, F *ibid* **51**, 336–340 (1933)
181 Laibach, F *ibid* **53**, 359–364 (1935)
182 Laibach, F and Fischnich, O *ibid* **53**, 469–477 (1935)
183 Laibach, F , Müller A M , and Schafer, W *Naturwissenschaften* **22**, 588–589 (1934)
184 Lane, R H *Am J Botany* **23**, 532–535 (1936)
185 Langham, D G *ibid* **28**, 951–956 (1941)
186 Larsen, P *Planta* **30**, 160–167 (1939)
187 Larsen, P 3-Indole-acetaldehyde as a growth hormone in higher plants Diss , Copenhagen (1944)
188 La Rue, C D *Am J Botany* **22**, 908 (1935)
189 La Rue, C D *ibid* **23**, 520–524 (1936)
190 Le Fanu, B *New Phytologist* **35**, 205–220 (1936)
191 van der Lek, H A A , Over de worteloorming van houtige Stekken Diss Utrecht (1925)
192 Levan, A *Hereditas* **24**, 471–486 (1938)
193 Levan, A *ibid* **25**, 87–96 (1939)
194 Link, G K K *Nature* **140**, 507–508 (1937)
195 Link, G K K , and Eggers, V *Botan Gaz* **101**, 650–657 (1940)
196 Link, G K K , and Eggers, V *ibid* **103**, 87–106 (1941)
197 Link, G K K , Eggers, V , and Moulton, J E *ibid* **101**, 928–939 (1940), **102**, 590–601 (1941)
198 Link, G K K , Wilcox, H , and Link, A *ibid* **98**, 816–818 (1937)
199 Linser, H *Planta* **31**, 32–59 (1940)
200 Lund, E J , *et al* Bioelectric Fields and Growth Univ of Texas Press, Austin (1947)

201 Mairold, O *Protoplasma* **37**, 445–521 (1943)
202 Marmer, D *Am J Botany* **24**, 139–145 (1937)
202a Michener, H D *ibid* **29**, 558–568 (1942)
203 Mitchell, J W *Botan Gaz* **101**, 688 699 (1940)
204 Mitchell, J W , and Martin, W E *ibid* **99**, 171–183 (1937)
205 Mitchell, J W , and Rice, R R Plant Growth Regulators *U S Dept Agr Misc Pub* No 495 (1942)
206 Mitchell, J W and Stewart, W S *Botan Gaz* **101**, 410–427 (1939)
207 Mitchell, J W , and Stuart, N W *ibid* **100**, 627–650 (1939)
208 Mitchell, J W , and Whitehead, M R *ibid* **102**, 393–399, 770–791 (1941)
209 Monselise, S P *Palestine J Botany* **5**, 106–111 (1945)
210 Morita, K *Bot Mag (Tokyo)* **32**, 39–52 (1918)
211 Müller, A M *Jahrb wiss Botan* **81**, 497–549 (1935)
212 Nagao, M *Sci Repts Tohoku Imp Univ* **12**, 191–193 (1937), **13**, 221–228 (1938)
213 Navez, A E *Proc Natl Acad Sci U S* **19**, 636–638 (1933a)
214 Navez, A E *J Gen Physiol* **16**, 733–739 (1933b)
215 Němeć, B *Vest Kral Ces Spol Nauk Ir* II, 1–34 (1934)
216 Nielsen, N *Planta* **6**, 376–378 (1928)
217 Nielsen, N *Jahrb wiss Botan* **73**, 125–191 (1930)
218 Nobécourt, P *Compt rend* **205**, 521–523 (1937)
219 Nobécourt, P *Bull soc botan France* **85**, 1–7, 490–493 (1938)
220 Nobécourt, P *Compt rend soc biol* **133**, 530–531 (1940)
221 Nobécourt, P *Rev Sci* **81**, 161–170 (1943)
222 Northen, H T *Botan Gaz* **103**, 668–681 (1941)
223 Nutman, P S , Thornton, H G , and Quastel, J H *Nature* **155**, 498–499 (1945)
224 Olson, R A and DuBuy, H G *Am J Botany* **27**, 392–401 (1940)
225 Oppenoorth, W F F *Rec trav botan Néerland* **38**, 289–372 (1942)
226 van Overbeek, J *ibid* **30**, 537–626 (1933)
227 van Overbeek, J *Proc Natl Acad Sci U S* **21**, 292 299 (1935)
228 van Overbeek, J *Botan Gaz* **100**, 133–166 (1938a)
229 van Overbeek, J *J Heredity* **29**, 339–341 (1938b)
230 van Overbeek, J *Plant Physiol* **13**, 587–598 (1938c)
231 van Overbeek, J *Botan Rev* **5**, 655–681 (1939a)
232 van Overbeek J *Proc Natl Acad Sci U S* **25**, 245–248 (1939b)
233 van Overbeek, J *Am J Botany* **28**, 1–10 (1941)
234 van Overbeek, J *ibid* **31**, 265–269 (1944)
234a van Overbeek, J *Ann Rev Biochem* **13**, 631–666 (1944)
234b van Overbeek, J *Econ Botany* **1** (1947)
235 van Overbeek J , and Bonner, J *Proc Natl Acad Sci U S* **24**, 260–264 (1938)
236 van Overbeek J , Gordon S A and Gregory, L E *Am J Botany* **33**, 100–107 (1946)
237 van Overbeek, J Olivo, G D , and Vasquez F M S *Botan Gaz* **106**, 440–451 (1945)
237a van Overbeek, J , Vasquez, E M S , and Davila, G *Botan Gaz* **106**, 440–451 (1945)
238 van Overbeek, J , and Went F W *ibid* **99**, 22–41 (1937)
239 Paál A *Jahrb wiss Botan* **58**, 406–458 (1919)
240 Pearse, H L *Imp Bur Hort Plantation Crops East Malling Kent Techn Commun* **12**, 88 pp , (1939)
241 Pich, B *Beih Bot Centr* **55**, 358–415 (1936)
241a Podešva, J *Acta Soc Sci Nat Moravicae* **14** (4), 1 24 (1942)

242 Pohl, R *Planta* **25**, 720–750 (1936)
243 Pohl, R *Ber deut botan Ges* **55**, 342–354 (1937)
244 Purdy, H A *Kgl Danske Videnskab Selskab, Biol Medd* **3**, 3–29 (1921)
245 Purvis, O N *Ann Botany* N S **8**, 285–314 (1944)
246 van Raalte, M H *Proc Konink Akad Wetenschappen Amsterdam* **39**, 261–265 (1936)
247 van Raalte, M H *Rec trav botan Néerland* **34**, 278–332 (1937)
248 Rappaport, J *Biol Jaarboek* **6**, 304–333 (1939).
249 Reed, H S, and Halma, F F *Univ Calif Pub Agr Sci* **4**, 99–112 (1919)
250 Reinders, D E *Proc Konink Akad Wetenschappen Amsterdam* **41**, 820 (1938)
251 Reinders, D E *Rec trav botan Néerland* **39**, 1–140 (1942)
252 Riker, A J, Berch, H, and Duggar, B M *Phytopathology* **31**, 19 (1941)
253 Robbins, W J *Bull Torrey Botan Club* **67**, 762–764 (1940)
254 Rogenhofer, G *Anz Akad Wiss Wien Math Naturw Klasse* **No. 11**, 1–2 (1936)
255 de Ropp, R S *Am J Botany* **34**, 53–62, 248–261 (1947)
256 Ruge, U *Planta* **27**, 352–366 (1937a)
257 Ruge, U *Biochem Z* **295**, 29–43 (1937b)
258 Ruge, U *Z Botan* **31**, 1–56 (1937c)
259 Ryan, F J, Tatum, E L, and Giese, A C *J Cellular Comp Physiol* **23**, 83–94 (1944)
259a Santen, A M A van Groei, Groeistof en Ph Diss, Utrecht (1940)
260 Scheer, B A *Am J Botany* **24**, 559–565 (1937)
261 Schmitz, H *Planta* **19**, 614–635 (1933)
262 Schneider, C L *Am J Botany* **25**, 258–270 (1938)
263 Schneider, C L *ibid* **29**, 201–206 (1942)
264 Schrank, F W *Plant Physiol* **19**, 198–211 (1944)
265 Schumacher, W *Jahrb wiss Botan* **82**, 507–533 (1936)
266 Schuringa, G J Diss, Utrecht (1941)
267 Segelitz, G *Planta* **28**, 617–645 (1938)
268 Sereisky, A C *J Inst Bot Acad Sci URSS Ukraine*, Nos **21–22**, 377–393 (1939)
269 Seubert, E *Z Botan* **17**, 49–88 (1925)
270 Shafer, J *Botan Gaz* **101**, 68–80 (1939)
271 Skoog, F *J Gen Physiol* **20**, 311–334 (1937)
272 Skoog, F *Am J Botany* **25**, 361–372 (1938)
272a Skoog, F, Schneider, C L, and Malan, P *ibid* **29**, 568–576 (1942).
273 Skoog, F, and Thimann, K V *Proc Natl Acad Sci U.S* **20**, 480–485 (1934)
274 Skoog, F, and Thimann, K V *Science* **92**, 64 (1940)
274a Skoog, F *Ann Rev Biochem* **16**, 529–564 (1947)
275 Slade, R E, Templeman, W G, and Sexton, W A *Nature* **155**, 497–498 (1945)
276 Smith, H H *Botan Gaz* **107**, 544–551 (1946)
277 Smith, P F *Am J Botany* **31**, 328–336 (1944)
278 Snow, R *Ann Botany* **39**, 841–859 (1925)
279 Snow, R *New Phytologist* **28**, 345–358 (1929)
280 Snow, R *Proc Roy Soc London* **B111**, 86–105 (1932)
281 Snow, R *New Phytologist* **34**, 347–360 (1935)
282 Snow, R *ibid* **35**, 292–304 (1936)
283 Snow, R *ibid* **36**, 283–300 (1937)
284 Snow, R *ibid* **37**, 173–185 (1938a)

285 Snow, R *ibid* **37**, 110–117 (1938b)
286 Snow, R *ibid* **39**, 177–184 (1940)
287 Söding, H *Ber deut botan Ges* **41**, 396–400 (1923)
288 Söding, H *Jahrb wiss Botan* **64**, 587–603 (1925)
289 Söding, H *ibid* **74**, 127–151 (1931)
290 Soding, H *ibid* **79**, 231–255 (1934)
291 Soding, H *ibid* **82**, 534–554 (1936)
292 Soding, H *ibid* **84**, 639–670 (1937a)
293 Söding, H *ibid* **85**, 770–787 (1937b)
293a Soding, H *Z Botan* **36**, 113–141 (1940)
293b Soding, H and Funke, H *Jahrb wiss Botan* **90**, 1–24 (1941)
294 Stark, P *ibid* **60**, 67–134 (1921)
295 Steward, F C, and Berry, W E *J Exptl Biol* **11**, 103–119 (1934)
296 Steward, F C, Berry, W E, and Broyer, T C *Ann Botan N S* **1**, 345–366 (1936)
297 Stewart, W S *Botan Gaz* **101**, 91–108 (1939)
298 Stewart, W S *ibid* **101**, 881–889 (1940)
299 Stewart, W S, Bergren, W R, and Redemann, C *Science* **89**, 185–186 (1939)
299a Stewart, W S, and Went, F W *Botan Gaz* **101**, 706–714 (1940)
300 Strong, M C *Mich State Coll Agr Expt Sta Quart Bull* **24**, 56–64 (1941)
301 Stuart, N W *Botan Gaz* **100**, 298–311 (1938)
302 Sweeney, B M *Am J Botany* **28**, 700–702 (1941)
303 Sweeney, B M *Am J Botany* **31**, 78–80 (1944)
304 Sweeney, B M, and Thimann, K V *J Gen Physiol* **21**, 439–461 (1938)
305 Sweeney, B M, and Thimann, K V *ibid* **25**, 841–854 (1942)
306 Tang, Y W and Bonner J *Arch Biochem* **13**, 11–25 (1947)
307 Templeman, W G, and Marmoy, C J *Ann Applied Biol* **27**, 453–471 (1940)
308 Templeman, W G, and Sexton, W A *Proc Roy Soc London* **B133**, 300–313 (1946)
309 Thimann, K V *J Gen Physiol* **18**, 23–34 (1934)
310 Thimann, K V *J Biol Chem* **109**, 279–291 (1935a)
311 Thimann, K V *Proc Koninkl Akad Wetenschappen Amsterdam* **38**, 896–912 (1935b)
312 Thimann, K V *Am J Botany* **23**, 561–569 (1936a)
313 Thimann, K V *Proc Natl Acad Sci U S* **22**, 511–514 (1936b)
314 Thimann, K V *Am J Botany* **24**, 407–412 (1937)
315 Thimann K V *Trans Intern Cong Soil Sci* **3rd Comm A**, 24–28 (1939a)
316 Thimann, K V *Biol Rev* **14**, 314–337 (1939b)
317 Thimann K V Currents in Biochemical Research Interscience, New York, 1946 Chap 21
317a Thimann, K V, and Behnke, J The use of auxins in the rooting of woody cuttings Harvard Forest, Petersham, Mass (1947)
318 Thimann, K V, and Bonner, J *Proc Natl Acad Sci U S* **18**, 692–701 (1932)
319 Thimann, K V, and Bonner J *Proc Roy Soc London* **B113**, 126–149 (1933)
320 Thimann, K V, and Bonner W D, Jr Unpublished data reported to AAAS meeting, Boston (1946), and *Am J Botany in press* (1948)
321 Thimann, K V, and Delisle, A L *J Arnold Arboretum* **20**, 116–136 (1939)
322 Thimann, K V, and Delisle, A L *ibid* **23**, 103–109 (1942)
323 Thimann, K V, and Koepfli, J B *Nature* **135**, 101 (1935)
324 Thimann, K V, and Lane, R H *Am J Botany* **25**, 535–542 (1938)
325 Thimann K V, and Poutasse, F F *Plant Physiol* **16**, 585–598 (1941)

326 Thimann, K V, and Schneider, C L *Am J Botany* **25**, 270–280 (1938a)
327 Thimann, K V, and Schneider, C L *ibid* **25**, 627–641 (1938b)
328 Thimann, K V, and Schneider, C L *ibid* **26**, 328–333 (1939a)
329 Thimann, K V, and Schneider, C L *ibid* **26**, 792–797 (1939b)
330 Thimann, K V and Skoog F *Proc Natl Acad Sci U S* **19**, 714–716 (1933)
331 Thimann, K V, and Skoog, F *Proc Roy Soc London* **B114**, 317–339 (1934)
332 Thimann, K V, and Skoog, F *Am J Botany* **27**, 951–960 (1940)
333 Thimann, K V, Skoog, F, and Byer, A *ibid* **29**, 598–606 (1942)
334 Thimann, K V, and Sweeney, B M *J Gen Physiol* **21**, 123–134 (1937)
335 Thimann, K V, and Went, F W *Proc Konink Akad Wetenschappen Amsterdam* **37**, 456–459 (1934)
335a Thimann, K V Unpublished results
336 Thompson, H E, Swanson, C P, and Norman, A G *Botan Gaz* **107**, 476–507 (1946)
337 Uhrová, A *Planta* **22**, 411–427 (1934)
338 Umrath, K, and Weber, F *Protoplasma* **37**, 522–526 (1943)
339 Veldstra, H *Enzymologia* **11**, 97–136 (1944a) 137–163 (1944b)
340 Veldstra, H, and Havinga E *Rec trav chim* **62**, 841–852 (1943) *Enzymologia* **11**, 373–380 (1945)
341 Voss, H *Planta* **27**, 432–435 (1937)
342 Voss, H *ibid* **30**, 252–285 (1939)
343 Wald, G, and DuBuy, H G *Science* **84**, 247 (1936)
344 Warner, G C, and Went F W Rooting of cuttings with indoleacetic acid and *Vitamin B*$_1$ *Plant Culture Publ Co, Pasadena, Cal, 1939*
345 Weaver, R J, Minarik C E, and Boyd, F T *Botan Gaz* **107**, 540–544 (1946)
346 Weij, H G van der *Rec trav botan Néerland* **29**, 379–496 (1932), **31**, 810–857 (1934)
347. Went, F W *Proc Konink Akad Wetenschappen Amsterdam* **30**, 10–19 (1926)
348 Went, F W *Rec trav botan Néerland* **25**, 1–116 (1928)
349 Went, F W *Proc Konink Akad Wetenschappen Amsterdam* **32**, 35–39 (1929)
350 Went, F W *ibid* **37**, 445–455 (1934a)
351 Went, F W *ibid* **37**, 547–555 (1934b)
352 Went, F W *Science* **86**, 127 (1937)
353 Went, F W *Bull Torrey Botan Club* **66**, 391–410 (1939a)
354 Went, F W *Am J Botany* **26**, 24–29 (1939b)
355 Went, F W *ibid* **26**, 109–117 (1939c)
356 Went, F W *Proc Konink Akad Wetenschappen Amsterdam* **42**, 581–591, 731–739 (1939d)
357 Went, F W *Botan Gaz* **103**, 386–390 (1941)
358 Went, F W *Plant Physiol* **17**, 236–249 (1942)
359 Went, F W *Botan Gaz* **104**, 460–474 (1943)
360 Went, F W, and Thimann, K V Phytohormones Macmillan, New York, 1937
361 Went, F W, and White, R *Botan Gaz* **100**, 465–484 (1939)
362 Wershing, H F, and Bailey, I W *J Forestry* **40**, 411–414 (1942)
363 White, P R *Bull Torrey Botan Club* **66**, 507–513 (1939)
364 White, P R, and Braun, A C *Cancer Research* **2**, 597–617 (1942)
364a Wildman, S G, Ferri, M G, and Bonner, J *Arch Biochem* **13**, 131–146 (1947)
365 Wildman, S G, and Gordon S A *Proc Natl Acad Sci U S* **28**, 217–228 (1942)

366 Wong, C Y. *Proc Am Soc Hort Sci* **36**, 632–636 (1938)
367 Wuhrmann-Meyer, K , and M *Jahrb wiss Botan* **87**, 642-678 (1939)
368 Yasuda, S *Japan J Genetics* **9**, 118–124 (1934)
369 Yasuda, S , Inaba, T , and Takahashi, Y *Agr and Hort* **10**, 1385–1390 (1935)
370 Zimmerman, P W *Torreya* **43**, 98–115 (1944)
371 Zimmerman, P W , and Hitchcock, A E *Contrib Boyce Thompson Inst* **8**, 337–350 (1937)
372 Zimmerman, P W , and Hitchcock, A E *ibid* **12**, 321–343 (1942)
373 Zimmerman, P W , and Hitchcock, A E *Proc Am Soc Hort Sci* **45**, 353–361 (1944)
374 Zimmerman, P W , Hitchcock, A E , and Wilcoxon, F *Contrib Boyce Thompson Inst* **8**, 105–112 (1936)
375 Zimmerman, P W , Hitchcock, A E , and Wilcoxon, F *ibid* **10**, 363–379 (1939)
376 Zimmerman, P W and Wilcoxon, F *ibid* **7**, 209–229 (1935)
377 Zimmermann, W A Z *Botan* **30**, 209–252 (1936)

Chapter III

Other Plant Hormones[1]

By KENNETH V. THIMANN

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The interrelationships between different parts of plants involve, in addition to the auxins, a number of other hormones Some of these have been studied in moderate detail, while of some the existence has only been inferred Because the work on any one hormone has been somewhat

[1] The author is much indebted to Dr F W Went for careful criticism of this chapter

isolated from that on others, each group will be treated in a separate section, with its own bibliography Interrelations with auxin, where these are indicated, will also be taken up in each section

I. Wound Hormones[2]

A Historical

When plants are injured, there typically results a stimulation of the growth of intact cells near the wound to produce scar tissue or "wound callus (*cf* 2) " This phenomenon involves the resumption of cell division by cells apparently fully mature More than fifty years ago Wiesner suggested that special substances may be produced by wounded cells which are responsible for this effect A series of investigations by Haberlandt and co-workers went far to confirm this view These experiments arose out of Haberlandt's first unsuccessful attempts to grow plant tissue cultures In small pieces of potato tuber, renewed cell division leading to formation of a periderm took place only if (*a*) a fragment of phloem tissue was present and (*b*) crushed cells, or an extract of them, were applied (16) Control of cell division was therefore ascribed to two hormones, one from the phloem, called "leptohormone" and one from wounded cells—the "wound hormone" proper The former was shown to be diffusible through agar and it may possibly be identical with auxin, though it has not been further studied In the kohlrabi root, cell divisions could be prevented by washing the injured surface, and could be induced by covering the surface with crushed tissue of other plants (17,19) Finally, by careful dissection, uninjured cells were exposed in the leaves of succulents and shown to respond by cell division to the application of tissue juices from other plants Reiche (26) obtained similar results by injecting petioles and stems with extracts of wounded tissue Hence the substances involved are not species specific

Search was made for suitable material for more extensive experiments, leading to the use by Wilhelm (40) of the parenchymatous lining of the hollow stem of the windsor bean, *Vicia faba*, and by Wehnelt (37) of the lining of the immature pod of the kidney bean, *Phaseolus vulgaris* This latter test has been adopted in later work

B Assay Method

The only method extensively used is that of Wehnelt (37), modified by Bonner and English (4) When the unripe beans are removed from the pod the parenchymatous tissue beneath is the responsive material A drop of the juice of crushed tissue (bean juice is very effective) applied to this layer causes a small intumescence a millimeter or two high to arise

[2] In this chapter each section has its own list of references

(Fig. 1). This consists of parenchyma cells elongating perpendicular to the axis of the pod and undergoing vigorous cell division. The height of

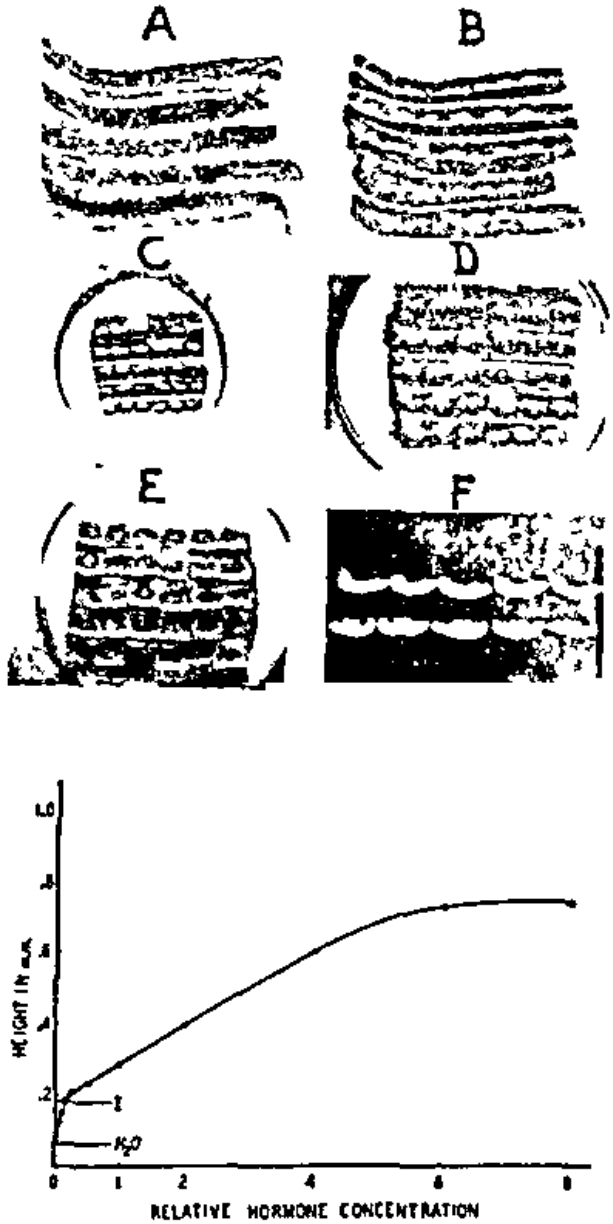


FIG 1.—Upper. Stages in the bean test A, fresh pods, B, pods slit and seeds removed; C, individual seed chambers in petri dish, D, drops of test solution in place, E, characteristic reaction to traumatic acid after 48 hours, F, cross section through seed chamber after 48 hours, top row, a control, lower row, reaction on the linear part of the curve

Lower: Relation between concentration and height of the intumescence Limit *of nonspecific effect* is shown at I (From Bonner and English, 4)

the intumescence after 48 hours, measured by a low-power microscope on a cross section, is proportional to the concentration of wound hormone There is, however, a small reaction, producing an intumescence of about

one fifth the maximum height, which is nonspecific in nature and may be caused by water, strong solutions of salts or sugars, toxic substances, etc This nonspecific effect was encountered by Wehnelt (37), Wilhelm (40), and Jost (18), who obtained reactions from such nondescript material as 2% levulose and 0 01% citric acid Such results have led to much confusion in the past The intersection point I in Fig 1 represents the highest nonspecific effect obtainable under the standard conditions, and its exact value varies from day to day Above this the intumescence is due to wound hormone alone The maximum height obtainable varies a good deal with the variety of bean, Bonner and English found "Kentucky Wonder, brown seed" the best The test is given (beyond the nonspecific point) by the juices of many plants, by molasses and brewers' yeast, but not by urine, peptone, or meat extracts The juice of the bean pod itself was, however, found to be the most active source, with brewers' yeast a close second There seems little support for the suggestion of Silberschmidt and Kramer (30) that activity of plant extracts on the bean increases with increasingly close taxonomic relationship to the bean

C Purification and Chemical Nature

Numerous pure amino acids, auxins, vitamins, and other biochemicals were found inactive (4) Indoleacetic acid was found by Jost (18) to be active at 1000 mg /l , but this was doubtless a nonspecific effect, due to toxicity It was also active, at 100 mg /l , on Wilhelm's test material (see above) In experiments by Orsos (23) on the kohlrabi root, tyrosine was found to be active It was inactive in the bean tests of Bonner and English Such differences suggest that different plants may have different limiting factors for the wound reaction, and that there may therefore be many substances interacting to produce the complete reaction

By extracting bean pod juice first with acetone and then with ethyl acetate at pH 2, extracting nonacid material with chloroform at pH 10, and forming barium salts, English, Bonner, and Haagen Smit (11) obtained a crystalline dibasic acid, $C_{10}H_{18}(COOH)_2$, which was active in the bean test The name proposed is "traumatin" or "traumatic acid," and the structure is apparently that of Δ^1-decene 1,10-dicarboxylic acid

$$HOOCCH{=}CH(CH_2)_8COOH$$

This was confirmed by synthesis (11) The yield was 18 mg from 100 lb fresh bean pods The activity was increased about 50% by addition of ½% sucrose (itself inactive) and was increased by a factor of two or more by adding some of the discarded acetone- and ethyl acetate-insoluble fractions (themselves of low activity only) This indicates that one or more cofactors varying in amount in the test beans, participate in the

reaction (see above) The most marked "cofactor" of this sort is glutamic acid, which at 0 25% (almost inactive alone) enhances the activity of the traumatic acid some ten times (10,36) As little as 0 1 γ traumatic acid, in the presence of a solution of cofactors, gives a detectable response in the bean test Furthermore the acid gives intense cell division in Haberlandt's test (15) on potato tubers (see introductory paragraph), and this too is enhanced by the cofactor solution

Apparently traumatic acid is only one of many closely related substances having wound hormone activity The saturated decane-1,10-dicarboxylic acid is about half as active as traumatic acid (12) The substances shown in Table I are all active to varying degrees according to English *et al* (10,12)

TABLE I

DICARBOXYLIC ACIDS OTHER THAN TRAUMATIC ACID ACTIVE AS WOUND HORMONES

Slight Activity	
Hexane-1,6- (suberic)	Heptane-1 7 (azelaic)
Activity About Half That of Traumatic	
Octane-1 8- (sebacic)	Decane 1 10
Active in Presence of Cofactor Solution	
Δ^1-Octene 1 8-	Δ^2-Tridecene-1 13-
Δ^1-Nonene 1 9-	$\Delta^{1\ 7}$ Octadiene-1 8-
Δ^2-Nonene-1 9-	5-Nonanone-1 9
Δ^2-Decene-1 10- (isomer of traumatic)	5-Nonanol 1 9-
Δ^4-Undecene 1 11	6-Undecanol 1 11
Δ^1-Tridecene 1,13-	6-Undecanone 1 11

Maleic acid showed very slight but definite activity, succinic acid none This fact and the activity of the pairs—octane- and octenedioic acids and decane- and decenedioic acids—indicate that unsaturation, while not essential, increases the activity Alcohol and ketone groupings in the chain do not remove the activity No monocarboxylic acid of a large number tested was active Activity appears, therefore, to be confined to dicarboxylic acids with a moderate number of carbon atoms in the chain

In a study of the substance which carries the stimulus when the sensitive plant (*Mimosa pudica*) is touched, shaken, or damaged, Soltys and Umrath (35) found that their partially purified preparations were also active in Wehnelt's bean test Study of other sensitive plants showed that activity on *Mimosa* could be separated from that on the bean test by chemical means, but activity on another plant, *Aeschynomene indica*, appeared to be brought about by the same substance as for the bean test The substance of Soltys and Umrath (36) was prepared from leaf extract by precipitation with lead and mercuric acetates and extraction with alcohol The final product appeared to be a dibasic hydroxy acid of

molecular weight about 420 with probably four acetylatable hydroxy groups An apparent nitrogen content of about 2% may be due to impurities, since English and Bonner also found nitrogen consistently in their semipure preparations Acetylation did not greatly reduce the activity It is not possible to conclude definitely whether the substance is one of those found active by English in the above test, the two hydroxy acids mentioned there are both of too small molecular weight Apparently final purification was not achieved

D Physiology and Interrelations with Auxin

It must be admitted that the physiology of wound growth is far from clear For one thing, a considerable part is played by auxin In woody plants, wound callus is produced at least in part by the vascular cambium, though Sharples and Gunnery (29) and Sass (28) have indicated that parenchyma of medullary rays is the main tissue whose division produces callus At any rate cambium typically responds to wounding by cell division and formation of new wood (8,9,14) Now this reaction can also be produced by auxin, as was first shown by Snow (31) for bean seedlings and by Soding (32,33) for trees (see 38, p 218, and Section VIII of the preceding chapter) The effect of pure indoleactic acid on poplar and willow was very striking, the new wood produced within 30 days being up to 1 mm wide (32) In white pine the new wood so formed is of the "rot-holz" type (39) Nevertheless this effect is limited to a region about 3 cm below the point of application of the auxin Also in the experiments of Rogenhofer (27) on the formation of callus at the base of poplar twigs, the effect of auxin was limited to a distance of about 3 cm below the point of application In the work of Wershing and Bailey (39) on "rot-holz" the effect of auxin was not transmitted very far down in young plants From a variety of experiments, however, we know that auxin is not limited to such short distances in its transport Indeed, the activation of cambium in the spring, by the developing buds, travels all the way down the trunk, taking many weeks to do so (7) Presumably this stimulus is (at least in part) auxin, and indeed it was shown by Avery *et al* (1) that auxin produced by the developing buds does in fact move down the shoot (apple trees were used) approximately parallel to the spread of the cambial activity It appears that within a very limited period in the spring even externally applied auxin can produce cambial stimulation over long distances, up to 23 cm , as shown by Gouwentak and Maas (15) with ash trees (*Fraxinus ornus*) If auxin can indeed travel long distances, at least in the spring and activate cambium, why then is the wound reaction of cambium limited to a few centimeters, and why is under most conditions the effect of applied auxin similarly limited?

Some light is thrown on this question by the work of Brown (5) By cutting incomplete rings with a bridge of bark remaining in the balsam poplar (*Populus balsamifera*), Brown showed that the wound wood was formed only weakly below most of the ring but was very strongly formed in a streamer below the bridge such as would be produced by a substance being transported polarly in the bark (Fig 2A) From this and other

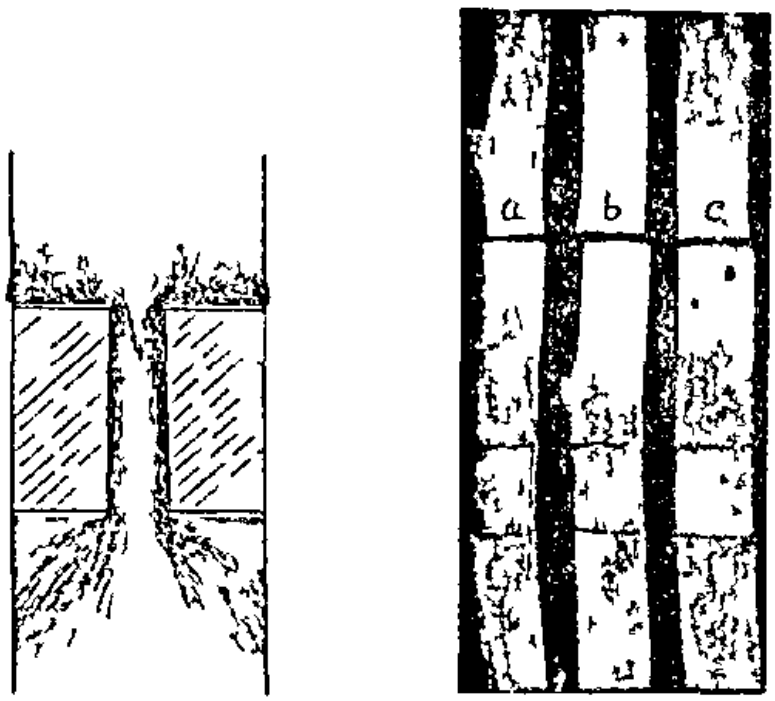


FIG 2 —A Cambial activity in relation to a longitudinally bridged ring as shown by xylem formation under the bark in *Populus balsamifera* The dotted lines indicate feeble cambial activity without differentiation of vessels or fibers (From Brown 5)

B Cambial activity in three units from the three-year old portion of one leader shoot of *Populus balsam fera* The upper (*a*) and lower (*c*) units were treated at the distal end (top) with indoleacetic acid 1 mg /g lanolin The middle (*b*) unit treated with lanolin only The longitudinal br dge (*cf* Fig 2A) in the lower sections is at the extreme right of each unit (From Brown and Cormack 6)

experiments he concluded that two factors are involved in the wound reaction the cambial hormone which moves basipetally downward in the phloem or cambium (33) (and is presumably auxin) and a wound substance whose effect is only local Brown and Cormack (6) showed that if the auxin is applied some 22 cm above the wound the wound reaction is much greater than without auxin but is still localized (see Fig 2B)

The cambium cultures of Gautheret (13) which continued to grow indefinitely on culture media as largely undifferentiated callus were con

siderably stimulated by traces of auxin added to the medium, presumably the act of cutting from the tree produced wound substances, enough at least to start the growth (see Section VIII, A of the previous chapter) In this connection it is worth noting that a crude bean extract, rich in traumatic acid, greatly promoted the growth of fragments of bean parenchyma in culture medium (3) The fragments did not grow indefinitely, however, so that no true tissue culture resulted All in all, it seems probable that the whole wound reaction involves in some way interaction of auxin with traumatic acid or other wound hormones Where auxin appears to have no effect, as in the bean pod, we may suspect it is already present in optimal concentration

There is a parallel for this in the case of root formation on cuttings here auxin applied at the base frequently has a greater effect if one side of the cutting is wounded This has been found by numerous horticultural workers, and especially by Rappaport (25) and La Rue (20) While it may be that the wounding improves the uptake of auxin, it seems unlikely that the effect can be due to this alone Soding's finding (34) that cambium scrapings from one plant (*Acer*) stimulate cambial activity in another (*Helianthus*) is also suggestive in this connection

Another important unknown is the biochemistry of the formation of traumatic acid As early as 1929 Petri (24) suggested that the wound hormone must be an oxidation product of a compound normally present in living cells The structure of traumatic acid would support this, and Nye and Spoehr (22) have pointed out that oxidation of C_{18} organic acids, particularly linolenic acid, could yield hexenal (which they isolated from *Ailanthus* leaves) and traumatic acid (see also 21) Certainly C_{18} acids occur in plants, but so little is known of the fatty acid metabolism of plant tissues that further discussion is valueless

REFERENCES

1 Avery, G S , Burkholder, P R , and Creighton, H B *Am J Botany* **24**, 51–58 (1937)
2 Bloch, R *Botan Rev* **7**, 110–146 (1941)
3 Bonner, J *Proc Natl Acad Sci U S* **22**, 426–430 (1936)
4 Bonner, J and English J Jr *Plant Physiol* **13**, 331–348 (1938)
5 Brown, A B *Can J Research* **C15**, 5–31 (1937)
6 Brown, A B and Cormack R G H *ibid* **C15**, 431–441 (1937)
7 Busgen M , and Munch F Structure and Life of Forest Trees Translated by T Thomson Wiley New York 1929
8 Coster, C *Ann Jard Bot Buitenzorg* **37**, 49–160 (1927)
9 Coster, C *ibid* **38**, 1–114 (1928)
10 English, J , Jr *J Am Chem Soc* **63**, 941 943 (1941)
11 English, J , Jr , Bonner, J and Haagen Smit, A J *Proc Natl Acad Sci U S* **25**, 323–329 (1939)

12 English, J , Jr , Bonner, J , and Haagen Smit, A J *J Am Chem Soc* **61**, 3434–3436 (1939)
13 Gautheret, R -J *Rev cyt cytophysiol vég* , **6**, 87–180 (1942–43)
14 Gouwentak, C A , and Hellinga, G *Mededeel Landbouwhoogeschool Wageningen* **39**, 1–6 (1935)
15 Gouwentak, C A , and Maas, A L *ibid* **44**, 1–16 (1940)
16 Haberlandt, G , *Sitzber kgl preuss Akad Wiss* 318–345 (1913), 1096–1111 (1914)
17. Haberlandt, G *Beitr allgem Botan* **2**, 1–53 (1921)
18 Jost, L *Ber deut botan Ges* **53**, 733–750 (1935)
19 Lamprecht, W *Beitr allgem Botan* **1**, 353–398 (1918)
20 La Rue, C D *Proc Natl Acad Sci U S* **27**, 388–392 (1941)
21 Meités M *Bull soc chim biol* **27**, 438–441 (1945)
22 Nye, W and Spoehr, H A *Arch Biochem* **2**, 23–35 (1943)
23 Orsos O *Protoplasma* **26**, 351–371 (1936)
24 Petri, L Cited in *Biol Abstracts* **7**, (5), 1015 (1933)
25 Rappaport, J *Biol Jaarboek* **6**, 304–333 (1939)
26 Reiche, H *Z Botan* **16**, 241–278 (1924)
27 Rogenhofer, G *Sitzber Akad Wiss Wien, Math -naturw Klasse Abt I* **145**, 81–99 (1936)
28 Sass, J E *Botan Gaz* **94**, 361–380 (1933)
29 Sharples, A , and Gunnery H *Ann Botany* **47**, 827–839 (1933)
30 Silberschmidt, K , and Kramer, M *Arquiv inst biol Sao Paulo* **7**, 125 (1936)
31 Snow, R *New Phytologist* **34**, 347–360 (1935)
32 Söding, H *Ber deut botan Ges* **54**, 291–304 (1936)
33 Söding, H *Jahrb wiss Botan* **84**, 639–670 (1937)
34 Söding, H *Z Botan* **36**, 113–141 (1940)
35 Soltys, A , and Umrath, K *Biochem Z* **284**, 247–255 (1936)
36 Umrath, K , and Soltys A *Jahrb wiss Botan* **84**, 276–289 (1936)
37 Wehnelt, B *ibid* **66**, 773–813 (1927)
38 Went, F W , and Thimann, K V Phytohormones Macmillan, New York, 1937
39 Wershing, H F , and Bailey, I W *J Forestry* **40**, 411–414 (1942)
40 Wilhelm, A *Jahrb wiss Botan* **72**, 203–253 (1930)

II. Flower-Forming Hormones

A Introduction

Unlike the hormones discussed above, flower-forming hormones or "florigens" have not been conclusively proved to exist Extracts or preparations from plants, having flower-forming activity and capable of transport in the plant, have never been obtained in spite of many efforts The evidence that a flower-forming hormone exists is thus indirect, although very strong, and it may be that flowering is controlled in some way by a balance between several substances

Although Sachs in 1880 had put forward the concept of special flower-forming substances which would cause the growing plant to change over from the production of leaves to that of flowers, the early workers in

general considered that flowering was dependent on the condition of the whole plant In 1907 Klebs developed evidence that flowering is induced by a low ratio of carbohydrates to soluble nitrogen, a view supported later by the work of Kraus and Kraybill (34) on the tomato Never thoroughly established, however, this conception was weakened by numerous subsequent workers, and was rendered untenable when Knodel (32) showed that in the same species, with a given carbohydrate nitrogen ratio, flowering may or may not occur while the plants may flower with very different values of this ratio

B Photoperiodism

The whole subject was put on a practical experimental basis by the discovery of Garner and Allard (22) that flowering is controlled by the length of day Some plants flower only when the day is shorter than a critical length (commonly ten hours or less), others only when it exceeds a critical length (commonly twelve or fourteen hours), while others again are essentially "day-neutral" It is not necessary that the prescribed length of day be maintained up to the time of flowering, frequently only a short treatment is necessary For example, plants of dill (*Anethum graveolens*) when grown in a day length ("photoperiod") of nine hours remained in a vegetative condition for eleven months, but, after exposure to four long days (eighteen to nineteen hours) and then return to the short days, they flowered in a few weeks Instead of the four photoperiods, continuous illumination lasting 84 hours would also cause flowering within a month (28) On the other hand, cocklebur (*Xanthium pennsylvanicum*), after growing vegetatively for many months on long days, could be induced to flower by treatment with a single short photoperiod The former is termed a "long-day" plant, the latter a "short-day" plant, in general the subsequent production of flowering by exposure to a particular series of photoperiods is called "photoperiodic induction"

Many other examples, and detailed discussion of the large volume of work on photoperiodism, may be found in the reviews of Garner (21), Tincker (56), Loehwing (39), Adler (1), Hamner (24,26), Burkholder (7) and two recent books (58,63)

There are sundry secondary effects The temperature prevailing during growth is of importance in some cases, the range of critical day length being a function of temperature, thus, to quote only one of many examples, *Baeria chrysostoma*, which requires long days for flowering, will not flower in long days or even continuous light if the temperature is above 25°C (54) Soybean, on the other hand, although a short-day plant, will not flower on short days if the temperature is too low (50) In one case, that of dill, a long-day plant, wounding of the stem or of roots

greatly increases the tendency to flower (48) Nutrition sometimes exerts a modifying influence, in barley (a long-day plant), nitrogen deficiency may induce flowering in spite of the photoperiodic conditions, that is, on a nine-hour day (3) Intensity of light may affect the actual length of the effective photoperiod, in the case of *Xanthium* 30 minutes will suffice if the intensity is high enough (26) These secondary points need not concern us here What is important, however, is that within a species, different varieties may have quantitatively different requirements In extreme cases the requirements may be almost opposite, thus, in tobacco (*Nicotiana tabacum*), the variety Samson will flower on long photoperiods while Maryland mammoth is a short-day plant

C Experimental Basis of the Hormone Concept

It was shown in 1925 by Garner and Allard (23) that, when only a part of the plant was exposed to photoperiodic induction, the stimulus to flowering need not be limited to that part When *Cosmos sulphureus*, a short-day plant, had the upper part completely darkened and the lower part exposed to short day, the lower part flowered, but on returning the whole plant to long days the upper part subsequently flowered Garner and Allard did not make any deductions as to the significance of this translocation, and the development of this line of approach into the hormonal concept was only initiated ten years later, first being foreshadowed by the experiments of Knott (33), and later established, in 1936, simultaneously by five investigators, Cajlachjan (8,9,10a) and Moshkov (46) in Russia, Kuyper and Wiersum (36) in Holland, and Melchers (41,42) in Germany

Cajlachjan's experiments with chrysanthemum, a "short-day" plant, were designed to study the importance of leaves in receiving the stimulus to flowering After some preliminary work on millet, which indicated that the response to change in day length depended on the amount of leaf surface exposed, he set up a large group of chrysanthemums of equal age and size The growing points and all the upper leaves and all lateral shoots except those in the upper part of the plants were removed, leaving, therefore, only leaves near the base and shoots near the apex They were then divided into four groups as follows group *1* received long day throughout, group *2* were also kept in long day, but the leaves were covered daily after ten hours, group *3* had the shoots covered daily after ten hours but the leaves were uncovered, and group *4* received short day (ten hours) throughout

Thus we have (*1*) leaves and shoots in long day, (*2*) shoots in long day, leaves in short, (*3*) leaves in long day, shoots in short, and (*4*) leaves and shoots in short day

In another similar series the shoots left on were those near the base, the leaves were those near the apex, and the four groups the same as above In both series, only the shoots of groups *2* and *4* flowered

Thus the photoperiodic stimulus is (a) received by the leaves and (b) transmitted along the petioles, the main stem, and the side shoots to the buds Cajlachjan (8) states [3]

"As in the processes of growth the regulatory function is performed by the hormone of growth, so in the processes of development this role is performed by a specific hormone of flowering The flowering of the plants and subsequent seed formation is due to the sufficient amounts of this hormone, which is formed in the leaves and translocated into the growing points'

Moshkov had been working on frost resistance He found (45) that white acacia can be prevented from freezing in the winter by subjecting it to short days in the latter part of the preceding summer Defoliated branches, however, could not be protected in this way Hence he came to consider that frost resistance, like flowering, is conferred by a photoperiodic stimulus received by the leaves His experiments on chrysanthemum (46) were similar to those of Cajlachjan, but more elaborate They confirmed the latter in showing that exposure of the buds alone to short day did not induce flowering Exposure of the leaves, but not the buds, to short day, induced flowering consistently Of the leaves, the two youngest were slightly effective, while the next four, *i e*, those young but fully developed, were the most effective in receiving and transmitting the stimulus This point was confirmed by Borthwick and Parker (5) for soybeans, in which the most effective leaf was found to be that which had most recently attained its full size The same workers (4) confirmed also that application of the photoperiod to the growing point alone does not initiate flowering, only the leaves can receive the stimulus

Among other interesting results, Moshkov showed that exposure of alternate leaves along the plant to short day did not induce flowering, so that there is an inhibiting effect exerted by those leaves which are in the long day This also has been confirmed by Borthwick and Parker (4) using soybeans with one branch in short day and one in long, the latter flowered only when it was defoliated

The experiments of Kuyper and Wiersum (36) were also with soybean (*Glycine max*, var Vilmorin) another short-day plant Two series of plants were grown, one in short days (9 5 hours) and one in long (thirteen to seventeen hours) Those in the long day produced no flowers

[3] The work of Cajlachjan, Moshkov, and others is most unfortunately largely published in Russian For careful and critical translations the author is much indebted to Miss K Zarudnaya Cajlachjan's conclusions, but not the main experiments, are set out in English (10a)

throughout the experimental period Apical parts of plants grown in short day, and already bearing flower buds, were grafted to bases of long-day plants, and the plants then maintained in long day After about seven weeks all the basal parts produced one to several flower buds Thus the flower-forming substance or stimulus was transported from the plant grown in short day, across the graft, to produce flowering in the part which had never received short day The experiments were later confirmed and extended (35), but were not successful with another variety, they believe that this is because with this variety the short-day graft continued to grow and blossom so freely in the long day that it used up all the flower-forming substance in itself

This latter phenomenon was noted by Cajlachjan (11) in very similar experiments with *Perilla nankinensis* The "hormone-donating" shoot, which had been given short day, was again grafted on to a "hormone-acceptor" stock which had had only long day When the donator had only leaves and the acceptor only shoots, the acceptor flowered freely But when the donator had shoots, or the acceptor had leaves, transference of the flowering stimulus was weak or absent Very similar results, but with the stock treated with short days and the scion in continuous light, were obtained by Moshkov (47a) (see also 27)

Melchers' experiments (41,42) were carried out with black henbane (*Hyoscyamus niger*), of which he used a biennial race *i e*, one which flowered only in the second year By grafting into the crown of one-year old plants, close to the growing point, a shoot of the two-year old, the growing point of the one-year old plant was caused to flower The material appears to graft very readily, and numerous variations of the experiment are possible (see below)

D Transport of the "Hormone"

In the experiments of Cajlachjan the flower forming stimulus traveled with apparently equal facility either up or down the stem, exposure of basal leaves caused flowering of apical shoots and *vice versa* This is in strong distinction to the movement of auxin, which (see Chapter 2, Section IV) under normal conditions travels in a strictly polar (basipetal) direction There are some indications that the flowering stimulus travels more readily down the stem than up Thus, in Moshkov's earlier work (45) on the frost resistance of acacia, the stimulus was found to travel downward from exposed branches into the trunk, but not upward Similarly, Kuyper and co-workers obtained upward movement of flowering stimulus (*i e*, stock on short day, scion on long) in only one plant out of 23, while the reverse movement took place in nearly all cases It must be remembered, however, that transport of the substance

is being deduced from observations of its effect In view of the opposing influence of the leaves on long day, shown in Moshkov s experiments above, and also in those on cocklebur by Hamner and Bonner (27), and confirmed by numerous others, transport upward could well occur without resulting in flowering Borthwick and Parker (5) found in soya that transport occurs equally in both directions, and the experiments discussed below all agree in this respect transport is not polar (see also 44,52)

It appears that transport may occur in any tissue except the wood Cajlachjan (11) showed with *Perilla*, a short-day plant, that, if the leaves which were given short day were separated from the buds by a section of stem in which a one sided cut was made, the buds still received the stimulus and subsequently flowered, indeed, the side of the shoot directly above the cut flowered just as soon as the opposite side This is taken to show transverse as well as longitudinal movement, but this deduction depends on the number of nodes between the stimulated leaves and the receiving buds However, he also showed that chrysanthemum leaves, of which the main vein was cut through, thus remaining attached to the stem only by parenchyma tissue at the base, could donate the flowering stimulus to buds on the main stem Although Lubimenko and Buslova (40) were unable to obtain this result with *Perilla ocymoides* Cajlachjan later (12) repeated it successfully on *Perilla nankinensis* There seems no doubt, therefore, that the "hormone" can travel in parenchyma

In the experiment with *Perilla* mentioned above, if instead of a one-sided cut the shoot was completely girdled, cutting all phloem, the stimulus was not transmitted In later experiments (12) the "hormone" was shown to move from one side to the other of a *Perilla* stem slit longitudinally all the way down to the base In this case transport was from the apical leaves down to the base through the bark, then transversely through cortical parenchyma and up again through the bark to the growing points of the lateral shoots

All the evidence therefore supports the view that the "hormone" travels in any direction in the plant, but only in living tissue Since living tissue is involved it is not surprising to find that local application of low temperature to the stem between the donating leaves and the receiving buds greatly delays transmission of the stimulus (6,13) Application of ether or chloroform to an internode also completely inhibited transport (13)

E Later Work on Hormonal Nature of the Stimulus

In the work discussed above, flowering has been envisaged as an 'all or-none" phenomenon either the plant forms flower buds or it does not A valuable step forward therefore, was made when Hamner (25)

introduced the measurement of the *number* of flower buds formed With this procedure he was able to show that, for a fixed cycle of nine hours light and fifteen hours dark, the effect, *i e*, the number of flower buds, is linearly proportional to the number of such cycles (see Fig 3) Such quantitative results very strongly support the hormonal nature of the stimulus By the same procedure it was also shown that both the light and the dark periods[4] are needed for completion of the flower-forming process in the short-day plants soybean and cocklebur This is not true for long-day plants, some of which, such as dill will flower in continuous light

The attempts made so far to extract an active hormone preparation have been suggestive but not convincing Hamner and Bonner (27)

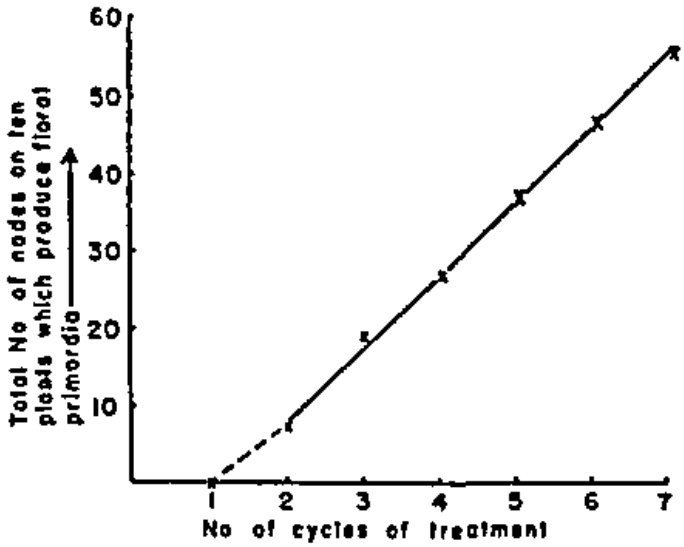


FIG 3 —Effect of number of cycles each consisting of a nine hour photoperiod and a thirteen hour dark period, on the number of floral primordia produced by Biloxi soybean (From Hamner, 25)

made grafting experiments with whole plants of *Xanthium* in which one plant with leaves was given short days, the other, defoliated, given long days The graft was of the veneer type, *i e*, both plants on their own roots After the graft had taken, the acceptors, *i e*, the plants on long day, flowered When the experiment was repeated, but with lens paper inserted in the graft, the long-day plants also flowered Unfortunately this latter experiment, which is crucial, could not be satisfactorily repeated, and Withrow and Withrow (59) have subsequently pointed out that, where transmission of the stimulus is observed, growth of tissue

[4] To parallel the term photoperiod Went has suggested 'nyctoperiod" (Gr *Nus, Nukti* = night) for the dark period The more exact meaning of darkness would be given by skotoperiod" (Gr *Skotos* = darkness) but, since this might lead to phonetic confusion nyctoperiod may be preferable

through the lens paper has occurred It is probable, therefore, that the successful result was due to a small amount of cellular connection A more striking claim was made in 1937 by Moshkov (47), who grew chrysanthemum under continuous illumination, removed the first four leaf blades, and attached to their petioles glass tubes filled with water Into these were inserted leaves from plants growing in short day and therefore containing the "hormone" Moshkov states

"No coalescence took place, nor could have done so, if only because the leaves were changed every day Even so, some of the chrysanthemum plants subjected to such treatment formed flower buds, whereas the control did not form any"

To the author's knowledge, no confirmation or extension of this most important experiment has been reported, nor is any unpublished work on this point mentioned in Cholodny's book (16) It only remains to be added that neither Hamner and Bonner (27), Sivori and Went (54), nor any other workers have obtained a flower forming effect with any combination of known growth substances or vitamins applied to leaves or roots, except in the pineapple (see Section J, page 96) However, the number of flowers may sometimes be increased by a variety of chemicals, in plants which are already flowering

About the only safe conclusion from these experiments is that the flower-forming material *may* pass outside the tissue, but it is not proved It seems certain, however, that quite small amounts of material are involved, and that small amounts of living tissue suffice to transmit it In some respects the data are suggestive of the behavior of viruses

F Specificity of the Material

Numerous experiments show that the "hormone" is not species specific, and, what is more important, that the flowering "hormones" of long-day and short day plants are the same Moshkov (47) used Samson tobacco, grown in continuous light, as hormone donator, and Maryland mammoth as acceptor Grafts of Samson on the latter caused it to flower in continuous light, provided only that the grafted scion was fairly large (25–30 cm long) Short scions (4 5 cm long) were inadequate, perhaps because they did not contain fully developed leaves (see above) Cajlachjan (11) similarly used sunflower (*Helianthus annuus*) as donator and artichoke (*Helianthus tuberosus*) as acceptor in grafting experiments and obtained good flowering in the latter Heinze *et al* (30) made numerous grafts of soybean varieties on one another, and obtained good transmission of the flower-inducing stimulus, particularly when the acceptor plant was defoliated Where single leaves were the donator, it was necessary for them to stay on for four days to cause flowering in the acceptor

More remarkable is the nonspecificity in Melchers' experiments (43), in which shoots, or even single leaves, of the short-day Maryland mammoth tobacco were grafted close to the growing point of one-year-old plants of *Hyoscyamus niger* Both plants are in the same family (*Solanaceae*), but separate genera The *Hyoscyamus* was thus induced to flower, but the curious result was obtained that it flowered equally whether the tobacco had been grown on long days or short This evidently means that even in long days the tobacco produces the "hormone," but is prevented from flowering either because there is not enough of it, because some other factor is needed as well or because an antagonistic substance is also present (see below) In later experiments a leaf of *Hyoscyamus* grown in long day, grafted on to the Maryland mammoth tobacco induced the latter to flower in long day Whatever the explanation of these phenomena, it is quite clear that the "hormone" is nonspecific

G The Light-Sensitive System

At least in the case of the long-day plant, it is evident that the "hormone" must be produced by light Considerable interest therefore attaches to the photosensitive system involved, particularly since it must be mainly present in mature leaves Moshkov from the first considered chlorophyll and the ordinary photosynthetic system to be responsible, and he explained the difference in effectiveness between young and mature leaves as due to differences in the amount or activity of chlorophyll But only recently has this view had any direct support

The first evidence that photosynthesis is involved came from the experiments of Parker and Borthwick (50a), who showed that carbon dioxide must be supplied to the plant in order for photoinduction to lead to flowering That this is due to the need for carbon dioxide in the actual photoinduction process, and not for the general life of the plant, was made clear by Harder and Witsch (29) using an individual leaf of *Kalanchoe* as hormone donator, and showing that carbon dioxide must be specifically supplied to that leaf, while it is on short day

As early as 1933 Rasumov showed that red light behaves like white for the photoperiodic effect, while blue and green act like darkness Withrow and Benedict (60) and Katunskij (31) confirmed this in general, though with some differences in regard to the effect of blue on certain long-day plants Funke (20), however, finds the effects of red and blue light are different for different plants More careful spectral studies using illumination of equal intensities (59) have shown that both in long- and short-day plants the longer wavelengths of the visible spectrum between 5770 and 7000 A are the most effective (see Fig 4) This

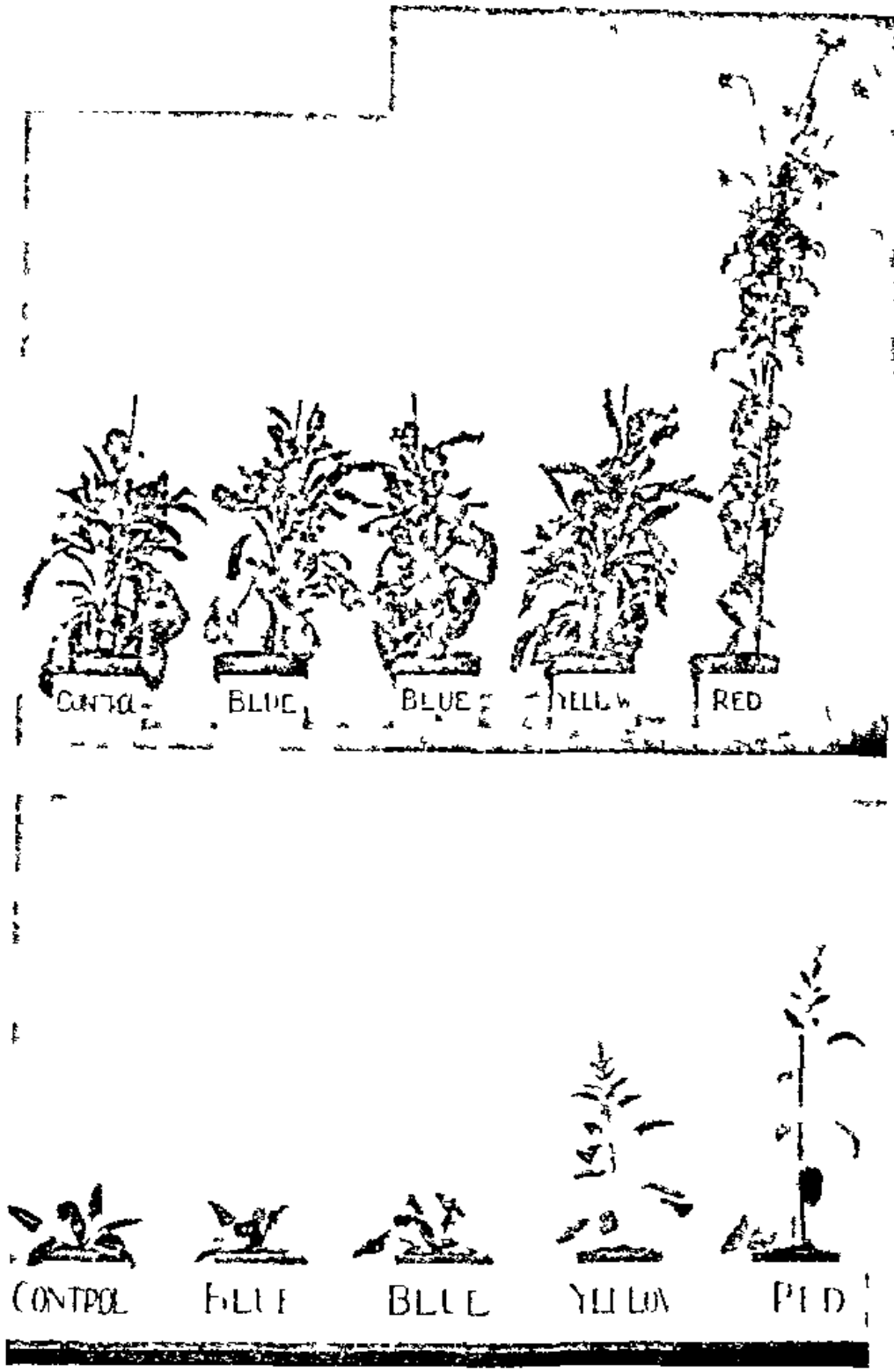


FIG 4.—Influence of color of light on flowering The light was given at 100 ergs/cm ^{2} (with blue also at 400 ergs/cm ^{2} in center pot) as supplement to natural day to make a total of 24 hours illumination

Above *Scabiosa atropurpurea* Scabious after 81 days below *Spinacia oleracea*, Spinach after 37 days (From Withrow and Withrow 61)

obviously suggests the spectrum of chlorophyll, and indeed Katunskij (31) specifically noted a secondary maximum in the blue and concluded that the effect of different wavelengths 'well correlates with spectra of chlorophyll absorption'

Recently Parker *et al* (51) have made a more thorough study with a specifically designed spectrograph to test this Instead of giving the whole illumination by selected spectral bands with all the accompanying

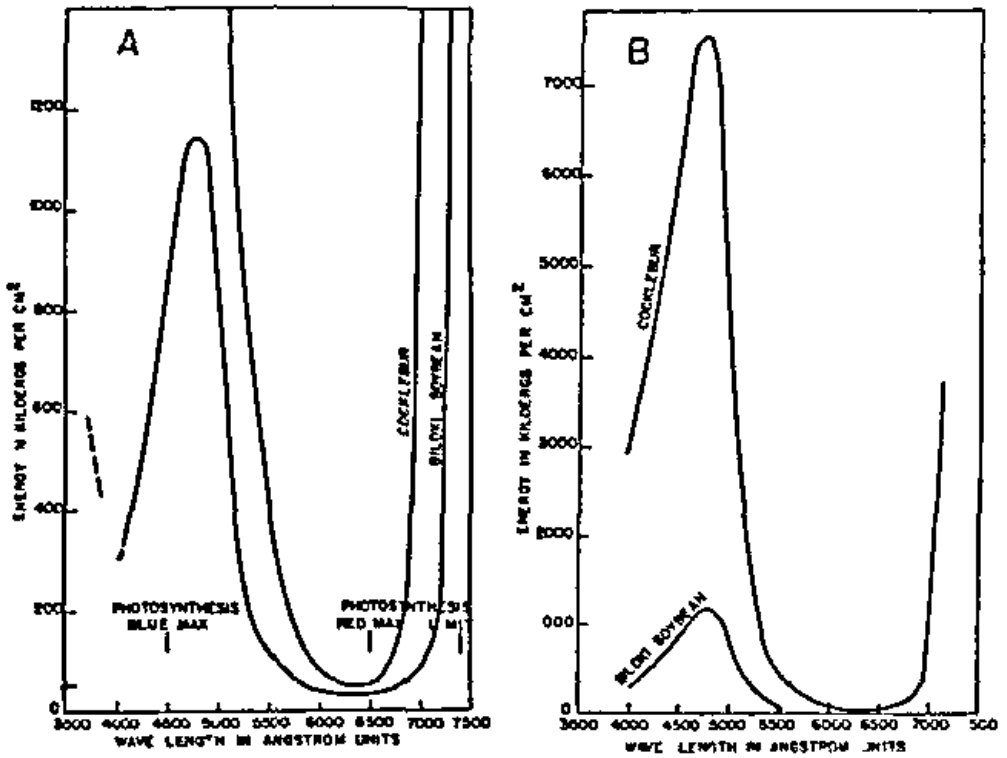


FIG 5.—Composite action spectrum for suppression of floral initiation in soybean (*Soja max*) and cocklebur (*Xanthium pennsylvanicum*) plotted on two different ordinate scales The soybean curve represents energy required at middle of fourteen hour dark period to prevent floral initiation the cocklebur curve represents energy similarly required at middle of twelve hour dark period (From Parker Hendricks Borthwick and Scully 51)

complications due to different amounts of etiolation and photosynthesis, they used the spectral bands to interrupt the dark period With Biloxi soybean and with cocklebur a brief interruption of the minimum dark period, providing this interruption occurs near the middle of the period, prevents flowering (see 25) The minimum energy needed to prevent initiation of flower buds is plotted against wavelength in Fig 5 The position of the cut off at the red end and the sharp drop between 4900 and 5400 A, are particularly suggestive, but the agreement with the chlorophyll spectrum at the blue end is not so good The tentative con

clusion is "the action spectrum is due to a porphyrin like material which is *probably* chlorophyll"

H Theoretical

Hamner (26) has put forward the following theory to explain in general terms the phenomena discussed above for short day plants (*1*) A substance or condition A is produced by light, its rate of production varies with temperature and with light intensity, and it decomposes slowly in darkness or in weak light In both short and long-day plants A increases up to a maximum with increasing time of illumination (*2*) A substance or condition B is produced in darkness, also increasing up to a maximum with increasing dark time Brief exposure to light destroys B at once (one minute's lighting during the dark period prevents flowering of *Xanthium*, 27) (*3*) When B reaches threshold concentration it interacts with A to produce the flowering hormone or flowering condition C The stability of C varies in different plants, as shown by differing degrees of transfer in grafting experiments etc The minimum dark period for flowering of short-day plants is thus the time needed to reach threshold concentration of B

The situation in long day plants is less clear Since some long-day plants can flower in continuous light, B would either have to be light stable in these plants or else conceivably not needed at all, *i e*, as soon as A reaches the threshold concentration C is formed

An entirely different theory for long-day plants is that of Melchers and Lang (44a) according to which the failure to flower in short days is due to the breakdown of some essential carbohydrate In *Hyoscyamus niger*, feeding of sugar allows flowering in short days, glucose, fructose, mannose, sucrose and maltose were equally effective Flowering was also induced in short days by placing the plants in pure nitrogen during the dark period, this, according to Melchers and Claes (44b) reduces the carbon dioxide production of the leaves The normal Pasteur effect, however would lead one to expect an accelerated carbohydrate breakdown in nitrogen It is conceivable, therefore, that the striking results of these workers may have another explanation

J Role of Auxin

The relation between auxin and flower formation is somewhat obscure In a general way auxin exerts an influence which opposes flowering For instance, conditions leading to vigorous growth, and presumably therefore to active auxin formation, tend to delay flowering An example is high nitrogen fertilization, which generally promotes vegetative growth and may delay flowering, see also Borodin's result (3) with low nitrogen given above In tobacco, high nitrogen promotes high auxin formation

in the stem tip (2), however, this is not the case in tomato (57), which shows no correlation at all between growth rate, auxin production, and added nutrients In oats, general nutrient deficiency (including nitrogen deficiency) hastened flowering (13a) but in millet it caused a slight delay

The most striking instance of the antiflowering action of auxin is given by the experiments of Dostál and Hosek (19) on *Circaea* In this plant isolated nodes from the apex will form flowers, those from the center will form leafy shoots, and those from the base storage organs The flowering of the most apical nodes is, however, as was long ago observed by Dostál, dependent on presence of the leaf If now the cut surface is treated with indoleacetic acid in lanolin ("auxin-paste") *flowering is completely inhibited, and the bud forms instead* either vegetative runners or tubers The experiments were carried out under presumably long-day conditions (Brno Czechoslovakia, in July) Here evidently the auxin has acted strongly against flowering Another experiment with auxin is unfortunately by no means so clear-cut Obsil (48a) reports that application of indoleacetic acid in lanolin to young shoots of *Lycopus* very strongly inhibited flowering, as compared to controls The shoots were halved longitudinally, each pair of opposite buds thus furnishing one treated and one control bud, in the same stage of maturity But since the criterion adopted was the actual opening of flowers, it is most probable that the effect was the normal inhibition of buds by auxin, which would be expected to occur, and which is discussed in Section VII of the preceding chapter An isolated fact which may prove significant is the observation of Zimmerman and Hitchcock (62) that triiodobenzoic acid applied to tomato plants causes axillary buds to develop into flowers *There is reason to believe (20a) that this substance* is an antagonist of auxin (in high concentrations) [5] since in soybeans it inhibits elongation and promotes lateral bud development, while it decreases auxin curvatures in the *Avena* test Treatment of soybeans with 200 mg /l triiodobenzoic acid increased the average number of flowerbuds from 3 2 to 36 2 However, it did not cause flowering on long days Galston (20a) concludes that there is normally antagonism between auxin and the flowering hormone

A very interesting and suggestive experiment of Sokolovsky (cited in 16) should be mentioned in this connection It will be remembered that in Moshkov's 1936 experiments, the plants in which alternate leaves along the plant were given short day did not flower Sokolovsky found that if these plants were decapitated they did flower A similar phenomenon was observed by Reece, Furr, and Cooper in the mango (53), in which removal of the terminal bud during the flowering causes the axillary

[5] *In lower concentrations triiodobenzoic* acid actually promotes the effect of auxin (54a)

buds, which would have remained vegetative or dormant, to differentiate into flowers Since the terminal bud is the major source of auxin in the plant, it might be suggested that removal of this source is enough just to turn the balance between auxin and flowering "hormone"

Defoliation acts in a similar way In *Hyoscyamus niger*, Lang and Melchers (38) obtained flowering on both short and long day when the plants were completely defoliated, one leaf regrafted and maintained in short day was enough to prevent flowering (37) Leaves are of course a source of auxin though not so powerful as the terminal bud

When seeds are treated with auxin and growth acceleration results (15,55) there is often a slight delay in flowering

A striking exception to this generally somewhat antagonistic effect of auxin to flowering is furnished by the pineapple Here a brief treatment with any one of several auxins (indoleacetic, naphthaleneacetic, and 2,4 dichlorophenoxyacetic acids in particular) induces flowering promptly and almost quantitatively (17,18,49) Some varieties respond only in certain seasons (18) others at all times and with a treatment of only 0 25 mg per plant (49) No other plant of all those used in the various types of auxin or of flowering experiment responds in this way, so that for the present this behavior must be regarded as quite exceptional

Cholodny, in his book (16), attempts to support the thesis that the flower-forming stimulus is exerted by a group of substances, one of which is auxin They are supposed to be effective only in certain specific proportions However, the possibility that auxin plays at least some part in promoting flowering had been considered by Cajlachjan and Zdanova (14), who made some experiments designed to show that leaves produce the most auxin under conditions in which they do not produce much flower forming "hormone" They diffused auxin from leaves into agar blocks and applied these to the outside of coleoptiles—a somewhat insensitive method—and the results so far as leaves are concerned, were inconclusive They did show clearly, however, with stem tips that auxin production increases with the duration of illumination, and that this is so for short-day (hemp chrysanthemum), long day (lupine, mustard) and day-neutral (sunflower) plants (14) Production of flower forming hormone of course, is not a simple function of illumination, and at least in short day plants must decrease with increasing illumination The fact that the mature leaves have greatest flower-forming effect, as mentioned above also shows that auxin is not the active agent, since mature leaves produce much less auxin than very young ones

We may conclude that auxin, if it plays any part at all in flower formation is in most plants an antagonist to the process Whether flowering results from a balance between the flowering "hormone" and auxin or other antagonistic substances is not proven as yet, but the

phenomena of flowering do strongly suggest that at least two factors are working in opposite directions, and that the difference between short- and long-day plants is due to differences in the relative rates of synthesis or destruction of these factors

REFERENCES

1 Adler, F *Forschungsdienst* 9, 332–367 (1940)
2 Avery, G S, Burkholder, P R, and Creighton, H B *Am J Botany* 24, 553–557 (1937)
3 Borodin, I *Bull Applied Botany, Genetics, Plant Breeding (U S S R)* 27, 171–195 (1931)
4 Borthwick, H A., and Parker, M W *Botan Gaz* 100, 374–387 (1938)
5 Borthwick, H A, and Parker, M W *ibid* 101, 806–817 (1940)
6 Borthwick, H A, Parker, M W, and Heinze P H *ibid* 102, 702–800 (1941)
7 Burkholder, P R *Botan Rev* 2, 1–52 97 168 (1936)
8 Cajlachjan, M H *Compt rend acad sci U R S S* 1, No 2, 85–89 (1936)
9 Cajlachjan M H *ibid* 3, No 9, 443–447 4, No 2 77 81 (1936)
10 Cajlachjan, M H *Izvest Akad Nauk S S S R* 3, 1093–1112 (1937)
10a Cajlachjan, M H *Compt rend acad sci U R S S* 16, No 4, 227–230 (1937)
11 Cajlachjan, M H *Izvest Akad Nauk S S S R* 6, 1249–1279 (1938) *Compt rend acad sci U R S S* 18, No 8 607–612 (1938)
12 Cajlachjan, M H *Compt rend acad sci U R S S* 27, No 2 161–163, No 3, 255–258 No 4, 373–376 (1940)
13 Cajlachjan, M H *ibid* 31, 949–952 (1941)
13a Cajlachjan, M H, and Lukovnikov, E K *ibid* 22, No 2 152–155 (1941)
14 Cajlachjan, M H, and Zdanova, L P *ibid* 19, 107 111 (1938)
15 Cholodny, N G *ibid* 3, No 1, 8, 9 (1936)
16 Cholodny, N G Phytohormones (in Russian) Akademia Nauk Kiev, 1939
17 Clark, H E, and Kerns, K R *Science* 95, 536–537 (1942)
18 Cooper, W C *Proc Am Soc Hort Sci* 41, 93–98 (1942)
19 Dostál, R, and Hosek, M *Flora* 31, 263–286 (1937)
20 Funke, G L *Rec trav botan Néerland* 40, 393–412 (1943).
20a Galston, A *Am J Botany* 34, 356–360 (1947)
21 Garner, W W *Botan Rev* 3, 253–276 (1937)
22 Garner, W W, and Allard, H A *J Agr Research* 18, 553–606 (1920)
23 Garner, W W, and Allard, H A *ibid* 31, 555–566 (1925)
24 Hamner, K C *Ann Rev Biochem* 13, 575–590 (1944)
25 Hamner, K C *Botan Gaz* 101, 658–687 (1940)
26 Hamner, K C *Cold Spring Harbor Symposia Quant Biol* 10, 49–59 (1942)
27 Hamner, K C, and Bonner, J *Botan Gaz* 100, 388–431 (1938)
28 Hamner, K C, and Naylor, A W *ibid* 100, 853 861 (1939)
29 Harder, T, and Witsch, H von *Naturwissenschaften* 29, 770–771 (1941)
30 Heinze, P H, Parker, M W, and Borthwick, H A *Botan Gaz* 103, 518–530 (1942)
31 Katunskij, V M *Compt rend acad sci U R S S* 16, No 8, 509–512 (1937)
32 Knodel, H *Z Botan* 29, 442–501 (1936)
33 Knott, J E *Proc Am Soc Hort Sci* (Suppl) 31, 152 154 (1934)
34 Kraus, E J, and Kraybill, H R *Oregon Agr Expt Sta Bull*, No 149, (1918)
35 Kuyper, J, and Schuurman, J J *Landbouwkund Tijdschr* 50 No 614 (1938)
36 Kuyper, J, and Wiersum L K *Proc Konink Akad Wetenschappen Amsterdam* 39, 1114–1121 (1936)

37 Lang, A *Naturwissenschaften* **30**, 590–591 (1942)
38 Lang, A, and Melchers, G *ibid* **29**, 82–83 (1941)
39 Loehwing, W F *Botan Revs* **4**, 581–625 (1938)
40 Lubimenko, V N, and Buslova, E D *Compt rend acad Sci U R S S* **14**, 149–152 (1937)
41 Melchers, G *Biol Zentr* **56**, 567–570 (1936)
42 Melchers, G *ibid* **57**, 568–614 (1937)
43 Melchers, G *Naturwissenschaften* **30**, 496 (1938)
44 Melchers, G *Umschau* **44**, 244–250 (1940)
44a Melchers, G, and Lang, A *Naturwissenschaften* **30**, 589–590 (1942)
44b Melchers, G, and Claes, H *ibid* **31** (1943)
45 Moshkov, B S *Bull Applied Botany, Genetics, Plant Breeding U S S R* Ser. III (6), 235–261 (1935)
46 Moshkov, B S *ibid* Ser A, Nos 17 and 19 (1936)
47 Moshkov, B S *ibid* No 21 (1937), *Compt rend acad sci U R S S* **15**, No 4, 211–213 (1937)
47a Moshkov, B S *Compt rend acad sci U R S S* **31**, No 7, 699–701 (1941)
48 Naylor, A W *Botan Gaz* **102**, 557–575 (1941)
48a Obsil, K *Planta* **29**, 468–476 (1939)
49 van Overbeek, J *Science* **102**, 621–622 (1945), *Rev agr Puerto Rico* **36**, 101–104 (1945)
50 Parker, M W, and Borthwick, H A *Botan Gaz* **101**, 145–167 (1939)
50a Parker, M W, and Borthwick, H A *ibid* **102**, 256–268 (1940)
51 Parker, M W, Hendricks, S B, Borthwick, H A, and Scully, N J *Science* **102**, 152–155 (1945) *Botan Gaz* **108**, 1–26 (1946)
52 Rasumov, V W *Bull Applied Botany, Genetics, Plant Breeding* Ser III, No 3 (1933)
53 Reece, P C, Furr, J H, and Cooper, W C *Am J Botany* **33**, 200–201 (1946)
54 Sivori, E, and Went, F W *Botan Gaz* **105**, 321–329 (1944)
54a Thimann, K V, and Bonner, W D, Jr *Plant Physiol* **23**, 158–161 (1948)
55 Thimann, K V, and Lane, R H *Am J Botany* **25**, 535–543 (1938)
56 Tincker, M A H *Sci Hort* **6**, 133–149 (1938)
57 Went, F W *Am J Botany* **31**, 597–618 (1944)
58 Whyte, R O Crop Production and Environment Faber and Faber, London 1946
59 Withrow, A P, and Withrow, R B *Botan Gaz* **104**, 409–416 (1943)
60 Withrow, R B, and Benedict, H M *Plant Physiol* **11**, 225–249 (1936)
61 Withrow, R B, and Withrow, A P *Plant Physiol* **15**, 609–624 (1940)
62 Zimmerman P W, and Hitchcock, A E *Contrib Boyce Thompson Inst* **12**, 491–496 (1942)
63 Vernalization and Photoperiodism a Symposium Chronica Botanica Co, Waltham Mass (1948)

III Leaf Growth Substances

As was mentioned in Chapter II, expansion of the leaf blade does not seem to be under the control of auxin, while growth of the veins probably is Growth of the blade is very sensitive to light, leaves of seedlings grown in complete darkness being always very small and unexpanded When equal energy exposures are given, the green region of the spectrum

is much less effective than the rest (15, and literature cited therein) The process is not, however, a simple function of photosynthesis, for Gregory (5) found in cucurbits that its temperature coefficient differs from that of photosynthesis, and deduced that a special photochemical reaction produces a substance which causes leaf expansion In plants growing on controlled photoperiods, the size of the leaves is often a function of the length of the photoperiod (7), though the night temperature is also a controlling factor Vyvyan (12) showed that leaf growth was dependent on the presence of cotyledons, and Went (13 14) confirmed and extended this, showing clearly that in the dark-grown pea seedling some factor or factors, stored in the cotyledons, controls expansion of the leaf blades Part of his results are summarized in Table II

TABLE II

LEAF AREA OF ETIOLATED PEA SEEDLINGS TEN DAYS AFTER OPERATIONS INDICATED

Condition of Plant	Total Area of First and Second Leaves Mm^2
Before treatment	24
Roots and cotyledons removed	24
Cotyledons removed	24
Roots removed	41
Intact	42

It is evident that the cotyledons, but not the roots, promote leaf growth Bonner, Haagen Smit, and Went (3) therefore examined the effectiveness of the diffusate from pea cotyledons in promoting leaf blade growth They used discs cut from the bases of young tobacco or radish leaves grown in the light The discs grew about 40% more in pea diffusate plus 1% sucrose than in the sucrose alone The reaction is independent of pH between 4 and 7 Certain amino acids, particularly proline and asparagine, and some purines, particularly adenine, were active (2), but the greatest increase of growth obtained was only about 20% Auxin, thiamin, and other vitamins were inactive Embryonic pea leaves showed a much greater effect when cultured in the pea diffusate (3) As shown in Fig 6, they reached a larger size on this medium in darkness than they would have done on the plant In experiments of the greenhouse type adenine was found to increase the leaf area of *Cosmos* plants grown in sand culture (2) It is of interest that adenine promotes the rooting of leaf cuttings (10) and that purines are known to be among the important nitrogenous constituents of leaves (11) Whether these substances really act as leaf growth hormones in the plant is, however, not proven In cultures of isolated stem tips of rye (*Secale*



FIG 6 —Growth of leaves excised from etiolated pea seedlings in culture solution after one month Top row in water alone middle row in inorganic salt medium plus 1% sucrose Bottom row in the same plus 1% standard pea diffusate solution (From Bonner Haagen Smit and Went 3)



FIG 7 —Left Tomato shoot with simplified leaves and enclosed growing point (+) Right Double leaf of tomato with fused petioles Both from buds treated with auxin (From La bach and Mai 6)



FIG 8 Leaves of *Cleome* Left Two leaves from control plants Right Five leaves from plants exposed to vapors of ethyl esters of 2 4-dimethylxyleneoxyacetic and α(2 4-dimethylxylenoxy) propionic acids (From Zimmerman *et al* 16)

cereale) on a sucrose–salts medium, De Ropp (4) found no promotion of growth of the leaf by pea diffusate or any other plant extract, nor by any vitamins, hence the situation in monocotyledons may be quite different Thus the whole problem remains in a suggestive, rather than a convincing, state

Although auxins do not appear to promote growth of the leaf blade in formed leaves, they do so in the rapidly developing leaf primordia This was first observed by Laibach and Mai (6), who showed that, when buds were treated with auxin, the subsequently developed leaves showed various abnormalities, including fusion of petioles and the growth of leaf tissue all round the growing point to enclose it like that of a monocotyledon (Fig 7) That auxin applied to buds actually increases the size of leaf primordia was shown by Snow and Snow (8) and Ball (1) Recently a number of experiments with the vapor of esterified auxins has been carried out by Zimmerman and co-workers, from one of whose papers (16) Fig 8 is taken (see Chapter 2, pp 17–21, 51) It shows clearly that leaf blade (mesophyll) tissue has extended laterally under the influence of the auxin Similar abnormalities were obtained by Ball (1) in *Tropaeolum*, the widening of the foliar primordia being particularly clear-cut and often leading to coalescence of two leaves at the base An extensive histological examination of this phenomenon will be found in the paper of Ball It is not easy to interpret such observations, embryonic leaves when damaged can regenerate their parts (9), so that some of these effects may be due to recovery after injury rather than to growth promotion proper In any event, such responses seem to be limited to very young primordia

REFERENCES

1 Ball, E *Am J Botany* **31**, 316–327 (1944)
2 Bonner, D M, and Haagen Smit A J *Proc Natl Acad Sci U S* **25**, 184 188 (1939)
3 Bonner, D M, Haagen Smit, A J, and Went F W *Botan Gaz* **101**, 128–144 (1939)
4 de Ropp R S *Ann Botany* N S **9**, 369–381 (1945) **10**, 31–40 (1946)
5 Gregory, F G *ibid* **42**, 469–507 (1928)
6 Laibach F and Mai C *Arch Entwicklungsmech Organ* **134** 200 206 (1936)
7 Lewis H, and Went, F W *Am J Botany* **32**, 1–12 (1945)
8 Snow, R, and M *New Phytologist* **36**, 1–18 (1937)
9 Snow, R, and M *ibid* **40**, 133–138 (1941)
10 Thimann, K V, and Poutasse, E F *Plant Physiol* **16**, 585–598 (1941)
11 Vickery, H B *Carnegie Inst Wash Yearbook* **24**, 349 (1925)
12 Vyvyan, M C *Ann Botany* **38**, 60–103 (1924)
13 Went F W *Plant Physiol* **13**, 55–60 (1938a)
14 Went, F W *Am J Botany* **25**, 44 55 (1938b)
15 Went, F W *ibid* **28**, 83–95 (1941)
16 Zimmerman, P W, Hitchcock, A E, and Harvill, E K *Contrib Boyce Thompson Inst* **13**, (5), 273–280 (1944)

IV. Vitamins, Steroids, and Carotenoids as Plant Hormones

Since vitamins are produced in plants, and since they take part in reactions of fundamental and quite general importance, it is hardly surprising that they should, to some extent, act as hormones in the plants in which they are produced The following is a very brief survey of the main aspects of their hormonal activity The early work has been reviewed by Bonner (3) and a full review published by Schopfer in 1943 (49), of which Chapters 6 and 7 are particularly pertinent

A Vitamins of the B Group

1 *Thiamin*

The early work of Robbins in 1922 (39,42) and Kotte (28) showed that isolated excised root tips will grow for a time in a medium containing only inorganic salts and sugar, but better when yeast extract or peptone is added By studying carefully the optimal concentrations of all constituents of the medium, White (1934) eventually was able to make continuous subcultures of tomato roots and thus to achieve "potentially unlimited growth"[6] The factor in yeast extract mainly responsible for the growth was shown simultaneously in 1937 by Bonner (2) for pea roots, and Robbins and Bartley (41) and White (56) for tomato roots, to be thiamin Isolated roots can be grown indefinitely in the salts-sugar medium with added thiamin, although their growth is not as rapid as with yeast extract (see below)

The discovery that thiamin is a growth factor for higher plants was actually made, before the work on root cultures, by Kogl and Haagen Smit in 1936 (27), who used isolated embryos of peas, freed from the cotyledons, cultivated in the dark on a nutrient gelatin medium They found that *biotin* greatly improved the growth of the shoot, but also that pure thiamin ("aneurin") at 0 01 mg /l increased both the length and the branching of the roots A selection of their results is given in Table III

The response of pea embryos to thiamin as well as other factors was further studied by Bonner and Addicott (9) Many tissue cultures, growing in light, appear not to require thiamin (Gautheret, 22,23) Roots, like many microorganisms, can utilize a mixture of the thiazole and pyrimidine moieties instead of the intact thiamin molecule Bonner

[6] For a complete discussion of plant tissue cultures see the reviews of White (55 58 60) and Gautheret (22 23)

TABLE III

GROWTH OF ISOLATED PEA EMBRYOS IN THE DARK ON SUCROSE—INORGANIC SALTS—GELATIN MEDIUM[a,b]

Addition, mg /l		Shoot, wt	Root, wt
Thiamin	Biotin		
0	0	92	47
0 0008	0	96	57
0 003	*0*	*107*	*61*
0 04	0	104	61
0 4	0	119	62
0	0 01	127	48
0 004	0 004	112	55
0 2	0 02	137	62

[a] Fresh weight in milligrams after eight weeks
[b] From Kögl and Haagen Smit (27)

(4) showed also that certain changes may be made in the molecular structure without impairing the availability of these compounds for growth A hydroxyl group in the thiazole, and the 6-amino group in the pyrimidine seem to be essential The requirements have been compared to those for numerous microorganisms in the review of Knight (26)

Evidence that thiamin promotes growth in plants does not necessarily establish it as a hormone, of course The hormone function of thiamin in the plant derives from our knowledge of its production and distribution The distribution of thiamin in the plant has been studied by the use of the fungus *Phycomyces blakesleeanus*, whose growth in a standard medium was shown by Schopfer (48) to be strictly proportional to the thiamin present The method was worked out by Schopfer and Jung (50) and applied to plant tissue by Rytz (46), Burkholder and McVeigh (17), and Bonner (6) With this method it has been shown that the growing apex has the highest concentration of thiamin, and that there is a gradient of concentration from the youngest to the oldest leaves Roots have a relatively low concentration, the thiamin is transported there from the leaves (15) The data of Burkholder and McVeigh (1940) for two varieties of corn (*Zea mays*) are summarized in Fig 9A, and those of Bonner (1942) on tomato in Fig 9B It is of interest that in these different plants the absolute concentrations are very similar, 20 γ/g dry weight in the tomato apex is about 0 07 millimoles/kg dry weight, while 60×10^{-3} moles/kg fresh weight in the corresponding tips

of the corn is about 0 06 millimoles/kg dry weight The relative concentration in the roots is, however, lower in corn than in tomato, averaging in fourteen hybrids only a quarter of that in leaves of medium age, while in tomatoes the value is about two thirds

These concentration data do not give any indication of direction of movement Bonner's experiments on girdling (7) show clearly, however, that the thiamin travels out of the mature leaves to the young leaves and

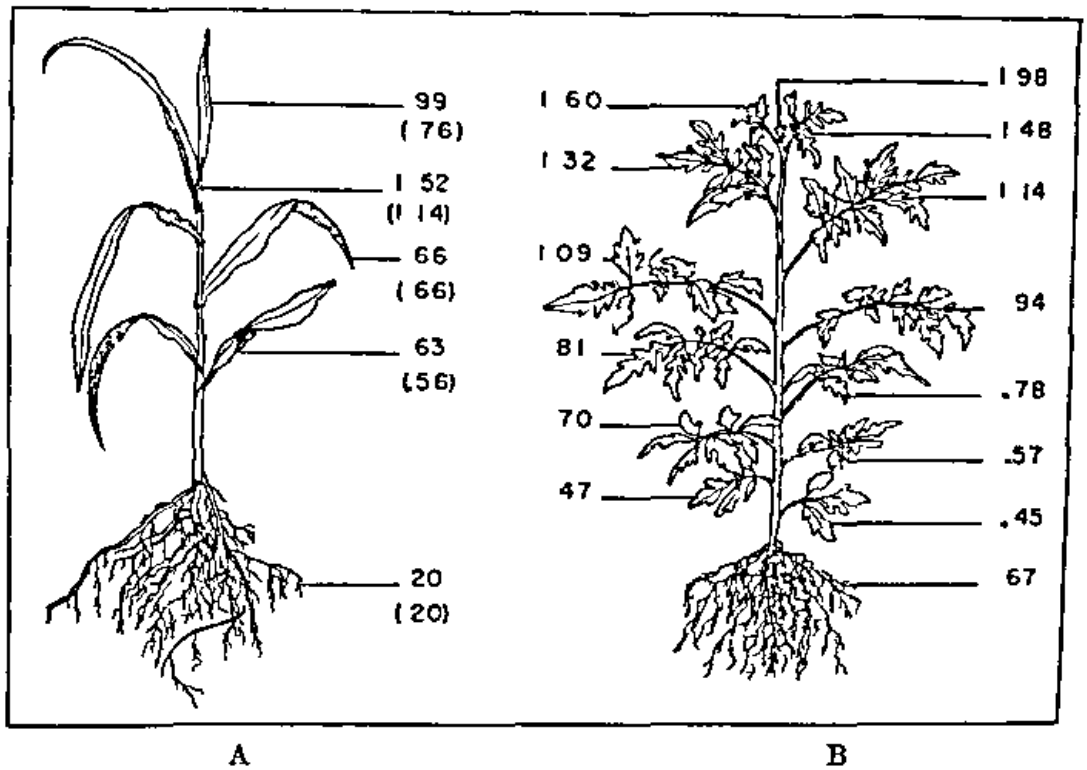


FIG 9 —Distribution of thiamin in leaves buds and roots expressed as γ/g fresh weight A corn data of Burkholder and McVeigh (17) The figures in brackets are determinations on another variety B tomato, data of Bonner (6) Bonner s data are given on a dry weight basis and have been corrected to fresh weight assuming 90 % water content

growing point and to the roots When the petiole of a mature leaf was girdled thiamin accumulated above the girdle, when the main stem was girdled just below the apex and the youngest leaves, it accumulated below the girdle When the main stem was girdled near the base (above the second node), however, thiamin accumulated above the girdle These data not only show the direction of movement, *i e* , from mature leaves to the growing apex and to the roots, but indicate that at least most of the transport of thiamin takes place in the cortex What the function of thiamin is in the growing leaves and terminal bud is not clear, but certainly in the roots it is essential for growth as discussed above

The actual function in roots is the same as in animal tissues, namely in decarboxylation of pyruvic acid Horowitz and Heegaard (25) have shown that the carboxylase of pea roots uses thiamin pyrophosphate as coenzyme The thiamin seems to be very closely linked to protein, during the action on pyruvate the enzyme loses much of its activity through the splitting off of pyrophosphate, but the thiamin remains protein-bound Thiamin is therefore a hormone produced in the leaves and transported to the roots to induce growth there, *i e*, a true growth hormone

2 *Pyridoxine*

In investigating the question as to why growth of isolated tomato roots was better when brown sugar was used instead of pure sucrose (the usual inorganic salts and thiamin being present), Robbins and Schmidt (43,44,45) studied the influence of various possible impurities in the brown sugar The ash was only very slightly beneficial, while amino acids and nicotinic acid were without effect but pyridoxine (vitamin B_6) had a large and immediate effect The average weight of roots in 50 ml of culture solution was raised from 3 4 mg with 5 γ thiamin to 16 1 mg with 5 γ thiamin plus 1 γ pyridoxine Robbins and Schmidt consider, therefore, that on thiamin alone the roots synthesize enough pyridoxine for slow, but not for maximum, growth Curiously enough, White (57) could not at first confirm this effect of pyridoxine either with his or with the Robbins and Schmidt strain of tomato Nevertheless, Bonner and Devirian (12) did confirm it with another strain, and Bonner (8) again found pyridoxine essential for growth with three clones of tomato root and also (5) for roots of sunflower (*Helianthus annuus*) Subsequently White in 1943 (59) did find an acceleration of growth when pyridoxine was used as supplement to thiamin in the tomato root clones of all three groups of workers

If it is accepted that pyridoxine is essential for root growth, at least in some plants, then data on the distribution and movement of this substance in the plant are needed to establish its hormonal nature It is evidently not synthesized in the roots themselves Bonner and Dorland (13), using a *Neurospora* mutant for bioassay of pyridoxine, find the highest concentration in the young (but not the youngest) leaves and a steady decrease throughout the older leaves There is also a gradient in the stem from apex to base, although the roots appear to contain more (14 γ/g) than the basal part of the stem (4–9 γ/g) Girdling experiments show, again, accumulation above a node near the base and below a node near the apex, also above a girdle in the petioles of mature leaves

It is evident, therefore, that pyridoxine is mainly produced in young but mature leaves (*cf* the flowering "hormone" discussed above) and transported both to the growing apex and to the roots Since it promotes growth at least in the roots, pyridoxine must be classed as a growth hormone

3 *Other Compounds*

The situation for the other vitamins of the B group is not so clear

Nicotinic acid was originally shown to be essential for pea roots and for tomato (12), but neither White nor Robbins and co-workers could at first confirm the effect Later, however, Robbins (40) and Bonner (8) showed that different strains or clones of tomato roots vary greatly in their need for nicotinic acid White (59) finds a small beneficial effect of nicotinic acid when glycine, thiamin, and pyridoxine are all present By analogy with other such cases, particularly among microorganisms it is probable that all roots require nicotinic acid for growth, but that many strains can synthesize sufficient for their needs As yet no data are available on the distribution and transport of nicotinic acid, but since some roots at least do not produce it in optimum quantities, it is likely that they will be stimulated by any which reaches them from the shoot, this would make nicotinic acid a sort of growth hormone, at least in certain strains of tomato and pea

Pantothenic acid shows a gradient of concentration from apex to base in the tomato plant according to Bonner and Dorland (14), but the concentration in the roots (29 γ/g dry weight) is about equal to that in the apex and youngest leaf (35 7 and 23 3 γ/g , respectively), so it is possible that it is synthesized in the roots In any event, it is not certain that there is a real requirement for pantothenic acid in roots or any other part, though a growth-promoting effect in the pea embryo has been reported (10) Its accumulation at girdles indicates transport similar to that of thiamin and pyridoxine Riboflavin, on the other hand, though showing a gradient of concentration from apex to base, did not accumulate much above girdles on the stem or on petioles, and Bonner (6) has found evidence that it is synthesized in root tips of tomato and four other plants

Biotin promotes growth in isolated pea embryos, especially of the shoot (Kogl and Haagen Smit, 27) (see Table III, above), and is evidently supplied to the growing seedling from the cotyledons, in which most of the biotin is stored Furthermore, biotin promotes the formation of roots in response to auxin, when ample auxin is supplied at the same time (see 54, Chapter XI) It has no effect on the growth of isolated oat coleoptiles In addition to the limited experiments with pure

biotin, Dagys (19) has made a number of determinations of the distribution of "bios II" The bios activity was determined on yeast growth It may be identical with biotin, or with biotin plus thiamin The bios II content of buds increases sharply in the spring when the buds begin to develop, and remains high during the summer in mature and growing leaves In the growing seedling it decreases in the cotyledons and increases in the embryo Thus although its activities are not entirely clear, biotin may well prove to be a plant hormone

In the above discussion attention has been centered on substances which behave as hormones in the strict sense of the word not merely as "growth factors" Thus ascorbic acid definitely promotes growth in isolated pea embryos (11) and in whole tobacco plants (20) and to a smaller extent in wheat (24), riboflavin promotes growth of eggplants (20) etc , but its role as a hormone is not clear The following two sections will summarize briefly a large quantity of experimental work whose significance for the hormonal control of growth and development is much more debatable

B Steroids

Accelerative effects of steroid preparations on plant growth have been claimed by numerous workers in the past fifteen years At first, the presence of auxin in many of the crude steroid preparations engendered doubts, but more recently clear cut effects have been obtained Pure estrone was shown to promote growth in the pea embryo by Kogl and Haagen Smit (27) and in other isolated embryos (10) Various investigators, especially Scharrer and Schropp (47), have found acceleration of flowering or growth promotion on treating whole plants or even fields of crops, with animal sex hormones However, many negative results have also been reported (see the reviews of Thimann, 52, Bonner, 3, and Bomskov, 1) Some of these may be due to lack of control of other conditions, for instance, Chouard (18) found that dihydrofolliculin (estradiol) accelerates growth and flowering of asters, but only when on an eight hour day, when given 15 to 22 hours of illumination no effect of the sterol was observed With *Fuchsia*, Burkhardt (16) found that high dosages of estrone only gave growth promotion when the "microelements" were added to the nutrient solution Lower estrone concentrations promoted growth and flowering under all conditions of mineral nutrition A clear acceleration of growth and increase in dry weight were obtained in three varieties of a grass, *Poa alpina*, by Zollikofer (61) Interestingly enough, Zollikofer subsequently found (63) that diethylstilbestrol is also active

in promoting vegetative growth, and for a given concentration appears somewhat more active then estrone This certainly suggests something in common between the effects on plant and animal tissue

If there is really a requirement of steroids for plant growth, then it is evident that plants vary a great deal in their ability to synthesize enough for their needs Although steroids do occur in plants, evidence that they are produced and transported as true hormones is wholly lacking Presence of steroids of the estrogen type was first shown in plant material by Loewe and Spohr (35), and by Dohrn *et al* (21) as early as 1926 There is some evidence for the occurrence of male hormones also (see Bomskov, *loc cit*)

At first it was thought possible that the steroid sex hormones might control sex in plants, but the effects observed can, with one exception, be ascribed to an influence on growth generally (see Zollikofer, 62) The exception, however, is provided by the interesting work of Love and Love (33,34) on various types of normal and intersexual flowers of *Melandrium dioecium* Crystalline estrone, estradiol, and estradiol benzoate, applied in lanolin paste to the axils of leaves in which flower buds would later develop, definitely shifted the subsequent flowers toward the female side, suppressing the development of anthers and promoting that of the gynecium Testosterone and its propionate had the opposite effect, promoting maleness These results apparently establish that animal sex hormones *can* control the sex expression of plants It remains to be seen of course whether such control is exerted by these substances under physiological conditions and in the concentrations normally present

C Carotenoids

Apart from their role in absorbing the light responsible for phototropic curvature, (see Section V of the previous chapter), the claimed hormonal effects of the carotenoids are few Lazar (32) found that carotene promotes root formation in *Impatiens* seedlings Such an effect has not been reported in other plants, and remains unconfirmed More remarkable are the experiments of Moewus (36) and of Kuhn, Moewus, and co workers at Heidelberg (29,30,31) According to this work, the unicellular green alga *Chlamydomonas eugametos* is controlled in many of its activities by the carotenoids crocetin and safranal and their derivatives which are excreted from the cells into the surrounding solution Crocin or crocetin gentiobioside, whose excretion is promoted by red light causes motility of the gametes Crocetin dimethyl ester causes copulation of these gametes and the sex affected depends on the previous irradiation of the solution There are eight sexes, from the strongest female through intermediate forms to the strongest male, and the copu

lation of each requires a specific period of irradiation with blue light This was traced to a conversion by light of the *cis* into the *trans* isomer Thus 95% *cis* and 5% *trans* activates the strongest females, 85% *cis* activates the next group, 75% the next, and so on, finally 5% activates the strongest males Further, safranal causes maleness and a glucoside of safranal, picrocrocin, causes femaleness The published results have certain inherent improbabilities, which are discussed by Philip and Haldane (38), Thimann (53), and Murneek (37), and, though Smith (51) did find a small effect of light in promoting copulation of gametes of three Californian strains, no other part of the work has been confirmed elsewhere The interpretation is made more complex, too, by the later finding (28a) that the activity of picrocrocin is probably due to an impurity of 10^5 times higher activity This substance, obtained from a *Crocus* species, appears to be a methyl ether of quercetin and thus quite unrelated to the above carotenoids An excellent summary of this work has been given by Lang (31a)

REFERENCES

1 Bomskov, C Methodik der Hormonforschung, Vol 2 Leipzig, 1939
2 Bonner, J *Science* **85**, 183 (1937a)
3 Bonner, J *Botan Rev* **3**, 616–640 (1937b)
4 Bonner, J *Am J Botany* **25**, 543–549 (1938)
5 Bonner, J *ibid* **27**, 811–821 (1940)
6 Bonner, J *ibid* **29**, 136–142 (1942a)
7 Bonner, J *Botan Gaz* **103**, 581–585 (1942b)
8 Bonner, J *Bull Torrey Botan Club* **70**, 184–189 (1943)
9 Bonner, J , and Addicott, F *Botan Gaz* **99**, 144–170 (1937)
10 Bonner, J , and Axtman, G *Proc Natl Acad Sci U S* **23**, 453–457 (1937)
11 Bonner, J , and Bonner, D *ibid* **24**, 70–75 (1938)
12 Bonner, J , and Devirian, P S *Am J Botany* **26**, 661–665, 667–671 (1939)
13 Bonner, J , and Dorland, R *Arch Biochem* , **2**, 451–462 (1943a)
14 Bonner, J , and Dorland, R *Am J Botany* **30**, 414–418 (1943b)
15 Bonner, J , and Greene, J *Botan Gaz* **100**, 226–237 (1938) **101**, 491–500 (1939)
16 Burkhardt, A *Ber Schweiz Botan Ges* **51**, 363–394 (1941)
17 Burkholder, P R , and McVeigh, I *Am J Botany* **27**, 853–861 (1940)
18 Chouard, P *Gynecologie* **34**, 253–257, (1935) *Compt rend soc biol* **126**, 509–512 (1937)
19 Dagys, J *Protoplasma* **24**, 14–91 (1935), **26**, 20–44 (1936)
20 Dennison, R *Science* **92**, 17 (1940)
21 Dohrn, M , Faure, W , Poll, H , and Blotevogel, W *Med Klinik* **22**, 1417–1419 (1926)
22 Gautheret, R -J *Rev Cytol Cytophysiol Végétale* **6**, 87–165 (1942–1943)
23 Gautheret, R -J La Culture des Tissus Gallimard et Cie, Paris, 1945
24 Havas, L *Nature* **136**, 435 (1935), **138**, 586 (1936)
25 Horowitz, N H , and Heegaard, E *J Biol Chem* **137**, 475–483 (1941)
26 Knight, B C J G *Vitamins and Hormones* **3**, 105–228 (1945)
27 Kögl, F , and Haagen Smit, A J *Z Physiol Chem* **243**, 209–226 (1936)

28 Kotte, W *Ber deut botan Ges* **40**, 260–272 (1922)
28a Kuhn, R, Low, I, and Moewus, F *Naturwissenschaften* **30**, 373, 407 (1942)
29 Kuhn, R, Moewus, F, and Jerchel, D *Ber* **71**, 1541–1547 (1938)
30 Kuhn, R, Moewus F, and Wendt, G *ibid* **72B**, 1702–1707 (1939)
31 Kuhn, R, and Moewus, F *ibid* **73**, 559–562 (1940)
31a Lang, A *Fortschr Botan* **11**, 268–317 (1944)
32 Lazar, O *Mem Soc Roy Sci Liège*, Ser IV, **1**, 3 (1936)
33 Löve, A, and Love, D *Svensk Botan Tid* **34**, 248–252 (1940)
34 Löve, A, and Love, D *Arkiv Botan* **32A**, No 13 1–60 (1945)
35 Loewe, S, and Spohr, E *Anz Akad Wiss Wien Math naturw Klasse* **63** 167 169 (1926)
36 Moewus, F *Jahrb wiss Botanik* **86**, 543–783 (1938), *Biol Zentr* **59**, 40–58 (1939), *ibid* **60**, 143–166 (1940)
37 Murneek, A E *Am Naturalist* **75**, 614–620 (1941)
38 Philip, U, and Haldane, J B S *Nature* **143**, 334 (1939)
39 Robbins, W J *Botan Gaz* **73**, 376–390, **74**, 59–79 (1922)
40 Robbins, W J *Am J Botany* **28**, 216–225 (1941)
41 Robbins, W J, and Bartley, M *Science* **85**, 246–247 (1937)
42 Robbins, W J, and Maneval, W *Botan Gaz* **76**, 274–287 (1923)
43 Robbins, W J, and Schmidt, M *Proc Natl Acad Sci U S* **25**, 1–3 (1939a)
44 Robbins, W J, and Schmidt, M *Bull Torrey Botan Club* **66**, 193–200 (1939b)
45 Robbins, W J, and Schmidt, M *Am J Botany* **26**, 149–159 (1939c)
46 Rytz, W *Ber Schweiz Botan Ges* **49**, 339–399 (1936)
47 Scharrer, K, and Schropp, W *Z Pflanzenernähr Dungung Bodenk* **13**, 1–9 (1934), *Biochem Z* **281**, 314–328 (1935), *ibid* **290**, 1–23 (1937)
48 Schopfer, W H *Ber deut botan Ges* **52**, 308 (1934)
49 Schopfer, W H Plants and Vitamins Chronica Botanica Co, Waltham, Mass, 1943
50 Schopfer, W H, and Jung A *Compt rend Ve Congrès Int Tech Chim Md Agricoles, Scheveningen*, 22–34 (1937)
51 Smith, G M *Am J Botany* **33**, 625–630 (1946)
52 Thimann, K V *Ann Rev Biochem* **4**, 545–568 (1935)
53 Thimann K V *Chronica Botan* **6**, 31 (1940)
54 Went, F W, and Thimann, K V Phytohormones Macmillan New York 1937
55 White, P R *Botan Rev* **2**, 419–437 (1936)
56 White, P R *Plant Physiol* **12**, 803–811 (1937)
57 White, P R *Am J Botany* **27**, 811–821 (1940)
58 White, P R *Biol Rev Cambridge Phil Soc* **16**, 34–48 (1941)
59 White, P R *Am J Botany* **30**, 33–36 (1943)
60 White, P R A Handbook of Plant Tissue Cultures J Cattell Press, Lancaster, Pa, 1943
61 Zollikofer, C *Ber deut botan Ges* **56**, 507–516 (1936)
62 Zollikofer, C *Scientia* Ser III **32**, 66–74 (1938)
63 Zollikofer, C *Schweiz Z Biochem* **1**, 1–9 (1942)

V. Additional Postulated Hormones

We have seen that, in the case of flower formation, the observations point strongly to the existence of a flower forming hormone or "florigen,"

but that proof of the existence of such a hormone has not been forthcoming In two other cases there is evidence for the functioning of a special substance or hormone, but proof of its existence has not been obtained These have been brought out by the work of Went, who has referred to the postulated substances as "calines'

A Rhizocaline

When in 1925 van der Lek (11) carried out his early experiments on root formation in cuttings, he postulated that the developing bud forms a hormone which moves downward in the cutting through the phloem and accumulates at the base, producing roots there Went later (20) found that the diffusate of leaves promoted root formation, and Bouillenne and Went (2) showed that the active substance is transported polarly from apex to base, it appeared to be stored in buds and cotyledons, and formed by leaves in light To this hormone they gave the name "rhizocaline' When it was subsequently found that the root-forming hormone was identical with auxin (see preceding chapter, Section VI), the conception of rhizocaline as a specific root forming factor was retained by Went (21,22), and the idea put forward that auxin causes root formation primarily by inducing the accumulation of rhizocaline in the basal zone of the cutting On the basis of experiments with hypocotyls of *Impatiens* seedlings, which form large numbers of roots without auxin and show very little increase when treated with any concentration of indoleacetic acid, Bouillenne and Bouillenne (1) insisted that auxin is not "the root-forming factor" In an extensive study of plant tissue cultures, Gautheret also concluded (8) that although root formation is due to hormones produced in buds, these hormones are not identical with auxin The experiments of Howard (10) on root formation in kale at first led him to the conclusion that auxin converts leaf initials into root initials, but he later showed that new root initials were formed very close to the auxillary bud Whether a shoot initial once formed can ever be *converted* into a root is thus not clear

It should, of course, be remembered that sucrose and thiamin are required for the roots to grow out, and in some plants also nicotinic acid and pyridoxine Thus auxin is certainly not the only factor controlling the formation of visible roots Indeed, in the kidney bean (*Phaseolus vulgaris*) Thimann and Poutasse (19) showed that a supply of available nitrogen, particularly potassium nitrate, asparagine, or adenine, promotes root formation much more strongly than does auxin, which presumably is present in nearly optimum concentration These materials exerted their effect partly by promoting the maintenance of the cutting, an effect which was also exerted by the leaves (see below) In *Impatiens*,

too, the amino acids glycine and alanine had an effect on the general maintenance of the hypocotyl cuttings (1) These substances, however, are essentially external factors There are clearly internal factors other than auxin involved in root formation Many plants do not root from cuttings even with optimum auxin treatment The peculiar fact that cuttings from young plants may form roots freely while cuttings consisting of tissues of the same age, but from older plants, do not do so was first noticed by Gardner (7) This was extended to various trees, especially pines and spruces, by Thimann and Delisle (18) They showed that this difference in rooting ability persists even in presence of optimum auxin treatment There is also a difference between the responses of different types of cuttings made from the same plant Recently van Overbeek and Gregory (15) studied the parallel case of rooting and non rooting varieties of the same plant Leafy scions of red ("rootable") *Hibiscus* were grafted to woody stocks of the white nonrooting variety and the resulting cuttings, after auxin treatment, formed roots readily This experiment strongly indicates that an internal transportable factor, coming from the leaves (*cf* 4,19), cooperates with auxin in root formation Indeed many workers have found a strong effect of leaves in promoting rooting of a variety of cuttings (see Section VII of the preceding chapter) On closer analysis (14) this factor supplied by the leaves of *Hibiscus* proved to consist of carbohydrate and nitrogen nutrients, and to be wholly replaceable by known substances, particularly sugar, ammonium sulfate, or arginine, in physiologically reasonable concentrations The concept of a "hormonal" factor, therefore, receives no support from this work

Evidence for the mobilization of rhizocaline by auxin treatment was brought by Cooper (3), who treated lemon cuttings at the base with 170 or 500 mg /l indoleacetic acid and after 15 hours cut off $\frac{3}{4}$ in of the base On now re-treating with auxin, very few roots were formed—in fact no more than when the bases were cut off without a re-treatment Controls from which the bases were not cut off rooted freely The portion removed is thus thought to have contained the rhizocaline However, Hellinga (9) Pearse (16), and Dorfmuller (6) repeated Cooper's experiments with various other plants and found no such effect Indeed, Cooper himself obtained this result only with certain auxin concentrations and times of treatment In Hellinga's experiments with *Coleus*, it was necessary to apply sugar to the cuttings Went (24) points out that in Pearse's willow cuttings most of the roots are formed from pre-existing primordia and not developed *de novo*, and shows that, in pea seedlings treated basally with 500 mg /l indoleacetic acid, cutting off the base and retreating does not produce as many roots as in controls treated first

with water To some extent the same treatment may be applied unwittingly when cuttings are treated basally with too high an auxin concentration For instance, Thimann and Delisle (18) showed that with blue spruce the treated base, which presumably would contain the mobilized rhizocaline, dies completely but roots are then formed above the dead portion

Somewhat more indirect though very suggestive evidence is given by Went's experiments (23) on root formation at the base and apex of auxin-treated seedlings When auxin is applied to dark grown pea seedlings at the apex, the location of the resulting roots depends on the auxin concentration used At low concentration the polarity of transport is normal, the auxin goes to the base, and all roots are formed at the base At high concentrations the transport system is overloaded or paralyzed (30) and some of the roots occur at the region treated, *i e*, at the apex When this happens, however, the number of roots at the base does not remain maximal but actually decreases In other words "the roots at the apex are formed at the expense of those at the base' (22) Went concludes, therefore, that the total number of roots is limited by a factor other than auxin

Phenylacetic acid is quite inactive for root formation in cuttings of etiolated pea seedlings, but such cuttings, if first treated with phenylacetic acid, afterward give an increased rooting response to auxin (24) This curious behavior is explained by Went in terms of the mobilization of rhizocaline by the phenylacetic acid, which in this respect is considered to act like a true auxin He thus envisages root formation as a dual process (*1*) the accumulation of rhizocaline at the base, which may be brought about by substances inactive or only weakly active as auxins, and (*2*) the activation of the rhizocaline, resulting in the formation of roots, this requires true auxins The only reasonable conclusion from all these experiments is that there probably is more than one internal "root-forming" factor, but the evidence that auxin "mobilizes" such material is as yet far from convincing

B Caulocaline

The experiments of Went (22,23), which indicate the storage of a leaf-forming factor in pea cotyledons, were discussed above (p 99) Very similar data were obtained which suggest the production in roots of a stem-forming factor Seedlings were decapitated and the stem length of the resulting lateral branches was measured The clearest experiment is shown in Table IV It is evident that stem growth is dependent on the roots, but not on the cotyledons The factor responsible for stem growth was termed "caulocaline"

TABLE IV

STEM LENGTH OF AXILLARY BUDS AFTER DECAPITATION AND PLACING BASES IN 2% SUCROSE[a]

Condition of Plants[b]	Stem Length of Buds, Mm
Cotyledons and roots removed	1 0
Roots removed	1 9
Cotyledons removed	21 2
Intact	26 3

[a] From Went (22)

[b] Dark grown plants, kept in dark throughout

The provision of sucrose solution obviates the possibility of carbohydrate as a limiting factor and goes some way toward eliminating the role of water The role of roots in promoting stem growth might, however, be due to improved water supply, as was suggested by de Ropp (5) in connection with his observation that stem tips of rye show greatly increased growth when they form roots A demonstration of increased stem growth without the participation of roots is therefore desirable This has been furnished by Went and Bonner (29), who cut off tomato stems at the base and kept them in darkness with various solutions applied to two of the leaves, the bases being in water Such stems grow little and do not respond to auxin appreciably, though they do grow after roots have been formed The application of coconut milk to one leaf, however, definitely increases stem growth (see Table V) The use of coconut milk was suggested by the finding of van Overbeek, Conklin, and Blakeslee (13) that this material promotes the growth of plant embryos in tissue culture Pea diffusate and, to a lesser extent, yeast extract or potassium nitrate solution were also active

TABLE V

ELONGATION OF TOMATO STEMS IN DARKNESS[a]

First leaf in	Second leaf in	Growth, mm[b]	
		First day	Second day
Water	Water	0	Dead
Sucrose 10%	Water	2 7	0 3
Sucrose 10%	Coconut milk 100%	4 2	2 9
Sucrose 10%	Coconut milk 50%	4 5	3 1

[a] From Went and Bonner (1943)

[b] Mean of six plants

Extracts of roots were, however, inactive This experiment certainly indicates that some factor besides auxin or sugar, though not necessarily

a hormone, is necessary for stem growth Another experiment of Went (26) goes far toward eliminating the factor of water supply as an explanation of the effect of roots on stem growth In this a part of the root system was submerged in nutrient solution, the other part allowed to grow in moist air Such plants showed greater stem growth than controls with the roots wholly immersed even though vigorously aerated Went concludes that the oxygen requirement for caulocaline production is greater than that for uptake of salts and water

In other experiments Went (27,28) has attempted to determine what are the limiting factors for growth of the entire plant Neither in peas nor in tomatoes is the ether-extractable auxin content of the tip correlated with general growth rate In tomatoes the water supply from the roots also does not limit growth In peas, in which different stem growth rates were obtained by means of different grafting combinations, Went concludes (23,27) that growth rate depends primarily on a factor coming from the stock, *i e*, stem base and root system, this factor is designated as the caulocaline

Strong evidence that roots are not *essential* for stem growth, however (though they appear to promote it), comes from two recent studies Loo (12) succeeded in growing isolated stem tips of asparagus in a simple nutrient medium and making apparently unlimited transfers These rootless stem tips grow indefinitely in light, though on the rare occasions when roots were formed the growth rate of the stem tips increased three- or four-fold, as was noted also by de Ropp (5) with rye stem tips The other is that of Skoog (17) with tissue cultures of callus formed by a tobacco hybrid, described and first cultured by White White showed (31) that these calluses, which grow as organless tissue when on the surface of solid media, readily produce stems when *immersed* in the culture solution, and Skoog's observations make clear that such stems are formed and elongate freely, quite independently of roots Roots indeed are very rarely formed, though occasionally a well-developed stem with leaves will give rise to a root Skoog concludes that no "caulocaline" is necessary for stem growth Internal factors may, of course, play an important part in controlling growth and differentiation "but in contrast with calines these substances must be present in all cells' (17) It is, of course, not excluded that they may be produced more vigorously in roots than in stems

Finally the proposed role of caulocaline in bud inhibition may be mentioned briefly As shown in Chapter II, pp 39–41, the application of auxin in place of the terminal bud causes the continued inhibition of development of the lateral buds Went (25) brought forward a number of experiments to show that this action is due to the mobilization of

caulocaline by the auxin, *i e*, it is accumulated at the point where the auxin is applied, so that none is available for growth But (as was described in Section VII, A of the preceding chapter) lateral buds may be inhibited when the auxin is applied directly on them, and not elsewhere on the stem, and isolated lateral buds growing in nutrient solution are strongly inhibited by auxin in the solution It is possible, of course, that such inhibition *in vitro* may not be the same phenomenon as inhibition of buds on the intact stem, but evidence for this is lacking Although the phenomena of inhibition are very puzzling, such facts make it difficult to invoke the mobilization of a bud growth factor to explain them

REFERENCES

1 Bouillene, R, and M *Bull soc roy bot belg* **71**, 43–67 (1938)
2 Bouillenne, R, and Went, F W *Ann jard bot Buitenzorg*, **43**, 1–178 (1933)
3 Cooper, W C *Plant Physiol* **11**, 779–793 (1936)
4 Cooper, W C *Botan Gaz* **99**, 599–614 (1938)
5 de Ropp, R S *Ann Botany* **9**, 369–381 (1945)
6 Dorfmuller, W *Jahrb wiss Botan* **86**, 420–490 (1938)
7 Gardner, F E *Proc Am Soc Hort Sci* **26**, 101–104 (1929)
8 Gautheret, R -J *Rev cyt cytophysiol vég*, **7**, 45–185 (1944)
9 Hellinga, G *Mededeel Landbouwhoogeschool Wageningen* **41**, 1–69 (1937)
10 Howard, H W *Ann Botany* N S **2**, 933–942 (1938), **4**, 589–594 (1940)
11 van der Lek, H A A Over de Wortelvorming van houtige steken Diss Wageningen, 1925
12 Loo, Shih-We *Am J Botany* **32**, 13–17 (1945)
13 van Overbeek, J, Conklin, M, and Blakeslee, A F *ibid* **28**, 647–656 (1941)
14 van Overbeek, J, Gordon, S A, and Gregory, L E *ibid* **33**, 100–107 (1946)
15 van Overbeek, J, and Gregory, L E *ibid* **32**, 336–341 (1945)
16 Pearse, H L *Ann Botany* N S **2**, 227–236 (1938)
17 Skoog, F *Am J Botany* **31**, 19–24 (1944)
18 Thimann, K V, and Delisle, A L *J Arnold Arboretum* **20**, 116–136 (1939)
19 Thimann, K V, and Poutasse, E F *Plant Physiol* **16**, 585–598 (1941)
20 Went, F W *Proc Konink Akad Wetenschappen Amsterdam* **32**, 35–39 (1929)
21 Went, F W *Biol Zentr* **56**, 449–463 (1936)
22 Went, F W *Plant Physiol* **13**, 55–80 (1938)
23 Went F W *Am J Botany* **29**, 44–95 (1938)
24 Went, F W *ibid* **26**, 24–29 (1939)
25 Went F W *ibid* **26**, 109–117 (1939)
26 Went, F W *Plant Physiol* **18**, 51–65 (1943)
27 Went, F W *Botan Gaz* **104**, 460–474 (1943)
28 Went, F W *Am J Botany* **31**, 597–618 (1944)
29 Went, F W, and Bonner, D M *Arch Biochem* **1**, 439–452 (1943)
30 Went, F W, and White, R *Botan Gaz* **100**, 465–484 (1939)
31 White, P R *Bull Torrey Botan Club* **66**, 507–513 (1939)

VI Hormone-Like Substances in Fungi

Compared to the amount of work on higher plants, the physiology of the fungi has been surprisingly little investigated Nevertheless, there

are a number of instances in which some process has been either postulated or proven to be controlled by a substance produced within the organism Most of these are connected with the sexual reaction The influence of externally applied substances, particularly vitamins, on sexual development or on the production of fruiting bodies will not be discussed here This work has been reviewed, together with all effects of vitamins on fungi, in the book by Schopfer (18)

The first evidence of the sort here considered was brought for members of the *Zygomycetes*, in which hyphae of + and − strains fuse to form zygospores at their point of contact on a solid medium As long ago as 1924, Burgeff (4) showed that in *Mucor mucedo* before the two mycelia come into contact, there is inhibition of elongation, followed by characteristic swelling and branching, which he considered as the initial stages in the sexual reaction By separating the + and − strains with a collodion membrane these effects were proved to be due to a diffusible substance (or substances), both strains being affected

Burgeff's findings were confirmed by Kohler (7) and also, with another organism, *Phycomyces blakesleeanus*, by Ronsdorf (16), who obtained evidence that, as might be expected, two diffusible substances were concerned, one produced by each strain The intensity of the sexual reaction was greatly increased by adding histamine to the medium Thiamin was shown by Schopfer (19) to have a similar effect on *Phycomyces*, while in *Melanospora destruens* Hawker (6) has shown that both thiamin and the balance between carbohydrates supplied control the formation of zygospores In a third organism, *Pilobolus crystallinus*, Krafczyk (8) again obtained similar results, showing clearly that, as Burgeff had indicated earlier, there are at least three distinct processes under hormonal control, namely, the branching and swelling ('telemorphosis"), the growth of special hyphae toward one another ("zygotropism"), and the delimitation of the gametangia

Very similar phenomena occur in the aquatic forms, and here progress has been much greater Couch in 1926 (5) observed some distance effects, corresponding to those of Burgeff, with *Dictyuchus monosporus*, but he could obtain no direct evidence for diffusible substances, the collodion membrane experiment being negative However, Bishop (1) with *Sapromyces reinschii*, obtained much clearer evidence and was able to cause increased branching in the tips of the hyphae of the male plant by adding the water in which the female plant had grown The extensive studies of Raper (1939–1942) with two species of *Achlya*, *A bisexualis* and *A ambisexualis*, include a similar experiment, as well as one with a cellophane membrane *à la* Burgeff From observations of this type, as well as from the rigid sequence of events in the sexual reaction, Raper

(10) deduced that four substances are involved, as follows Hormone A, produced by the female plant, which starts the reaction by inducing the formation of antheridial branches near the tips of the male hyphae (*cf* "telemorphosis," above), Hormone B, produced by the male plant after the above reaction, causing the formation of oogonial initials on the tips of the female hyphae, Hormone C, produced by the oogonial initials (and not by other hyphae of the female plant), which causes the antheridial hyphae to grow toward these initials (*cf* "zygotropism," above), and also induces the delimitation at their tips of the male gametangia, or antheridia, and Hormone D, presumably produced by the antheridia, which causes delimitation of the oogonia from their stalks, and subsequent development of the oospheres Since this stage takes place usually after direct contact with the antheridia, the evidence that it is controlled by a diffusible substance or hormone is not fully convincing

The existence of at least the first three substances was pretty well proved by exposure of plants at the appropriate different stages of development to diffusates from cultures of the opposite sex The two *Achlya* species evidently use and produce the same hormones, though the production rates and sensitivities are different However, chemical experiments so far are limited to Hormone A Using a standardized measure of antheridial branch formation, Raper (11) obtained temperature, pH, and concentration curves, and discovered a marked, but irregular, diurnal periodicity in the response Addition of $2\ 10^{-4}$ *M* malonic, glutaric, or pimelic acid greatly increased the production by the female plant Concentration of Hormone A from large-scale cultures by Raper and Haagen Smit (12) through many stages led to a 70,000 times enrichment, but not to a pure preparation It was concluded that the substance is a neutral ketone, and is active in a concentration of 1 in 10^{12} Activity is destroyed completely by 2,4-dinitrophenylhydrazine, and partially by the reagent of Girard and Sandulesco A number of barbiturates showed activity, but only at relatively high concentrations Further chemical work will be awaited with great interest

A reaction of another kind is that of the aggregation of individual amebae into a fruiting body, one of the stages in the life cycle of the *Acrasiales* The spores of these organisms germinate into myxamebae which grow and multiply for a time, feeding on bacteria, and then suddenly flow together into a sort of mound, termed a pseudoplasmodium In *Dictyostelium discoideum* the life cycle has been worked out in detail by Raper (13,14,15) and Bonner (2), who have considered the aggregation stimulus to be chemical in nature This was virtually proved by the experiment of Runyon (17), who placed a cellophane membrane over an aggregating mass of myxamebae and found that additional myxamebae

above this would follow the aggregation of the pattern below Bonner (3) has carried out many similar experiments, particularly with aggregation under water, and concludes that aggregation is due to the gradient of a substance, "acrasin," produced by all myxamebae, but unstable enough to be constantly breaking down, so that the gradient is maintained No chemical work has yet been carried out The phenomena of polarity and dominance observed in the aggregation are in many ways suggestive of those due to auxin in higher plants

REFERENCES

1 Bishop, H Thesis, Harvard Univ , Cambridge, Mass (1937)
2 Bonner, J T *Am J Botany* **31**, 175–182 (1944)
3 Bonner, J T Thesis, Harvard Univ , Cambridge, Mass (1947)
4 Burgeff, H *Botan Abhandl* **4**, 5–135 (1924)
5 Couch, J N *Ann Botany* **40**, 848–881 (1926)
6 Hawker, L E *Ann Botany* N S **3**, 455–468, 657–676 (1939)
7 Kohler, F *Planta* **23**, 358–378 (1935)
8 Krafczyk, H *Beitr Biol Pflanzen* **23**, 349–396 (1935)
9 Raper, J R. *Am J Botany* **26**, 639–650 (1939)
10 Raper, J R *ibid* **27**, 162–173 (1940)
11 Raper, J R *ibid* **29**, 159–166 (1942)
12 Raper, J R , and Haagen Smit, A J *J Biol Chem* **143**, 311–320 (1942)
13 Raper, K B *Am J Botany* **27**, 436–448 (1940)
14 Raper, K B *J Elisha Mitchell Sci Soc* **56**, 241 282 (1940)
15 Raper, K B *Growth* (Suppl) (3rd Growth Symposium) **5**, 41–76 (1941)
16 Ronsdorf, L *Planta* **14**, 482–514 (1931)
17 Runyon, E H *Collecting Net* **17**, 88 (1942)
18 Schopfer, W H Plants and Vitamins Chronica Botanica Co Waltham Mass (1943)
19 Schopfer, W H *Bull soc botan suisse* **40**, 87–111 (1931)

Chapter IV

Hormones in Insects

By BERTA SCHARRER

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I. Introduction

The study of insect hormones represents a particularly active sector of the wide and relatively young field of invertebrate endocrinology (see reviews 13,20,21,35,44,54,67,69,71,85,88,95,98,118,124,135,138,159,173, 183) In insects endocrine factors are known to play an important role in reproduction and in postembryonic development By comparison the hormonal control of color change is of minor significance The question as to whether sex hormones comparable to those of vertebrates are operative in the insect organism is still controversial Finally, there exist in this group of invertebrates physiologically active substances which participate in the nonautonomous development of hereditary characters and which, because of their similarity with hormones in the commonly accepted sense of the word, have been termed "gene hormones"

With regard to the relationship between the endocrines of vertebrates and those of insects, only few conclusive data are available These indicate that, in principle, vertebrate hormones may act on insects, and insect hormones on vertebrates Details on this subject may be found in several monographs (71,88,173, see also 104b)

The actions of insect hormones are being studied by various methods: extirpation and implantation of endocrine organs, injection of organ extracts, denervation of endocrine glands, ligatures, blood transfusions, parabiosis, etc. Most of these methods are used in vertebrate endocrinology and are applied to insects with only minor modifications. One of the newly developed techniques is of interest. Test organs such as skin, gonads, etc., with or without endocrine glands, are implanted into the abdominal cavity of hosts whose own physiological condition offers a "neutral" endocrine surrounding preferable to any tissue culture medium (15,170).

II. Endocrine Control of Postembryonic Development

Among the physiological processes under hormonal control in insects, the most extensively studied is postembryonic development, which, in all groups except the Ametabola, consists of a series of developmental steps leading from the larva or nymph, newly hatched from the egg, to the adult insect.

In the holometabolous insects, such as butterflies, periodic steps of growth as evidenced by molts produce larval forms (instars) of increasing sizes. The larval period is terminated by pupation, which marks the onset of adult differentiation of tissues and organs, although "internal" metamorphosis may begin during the larval period (170). Metamorphosis is completed at the end of the pupal stage when the adult form (imago) emerges.[1]

In hemimetabolous insects, for example grasshoppers, the immature forms or nymphs likewise undergo a number of molts. Each molt produces a nymph which is not only larger than the preceding instar but is a step closer to the adult form. In this group of insects with "incomplete" metamorphosis a pupal period is lacking, but during the last nymphal stage considerable morphological changes occur which at the final molt result in the fully developed imago.

Quite generally then, in normal development the larval (nymphal) period of an insect is predominantly one of growth. Little adult differentiation occurs before the insect has reached the appropriate stage for metamorphosis. Accordingly larval molts mark a step in growth rather than in imaginal differentiation. Within certain limits molts may occur as long as the organism remains immature, they cease to take place as soon as metamorphosis is completed. This statement holds true in spite of the fact that under certain experimental conditions it has been possible to induce adult skin to molt again (115,120,182,184).

[1] Hypermetamorphosis is not dealt with in this chapter since no experimental data are available concerning this phenomenon.

For the understanding of hormonal regulations of development it is useful to point out that there exist two types of molts (1) larval (nymphal) molts in which an increase in size but little or no imaginal differentiation occurs, (2) molts coupled with imaginal differentiation, for instance the final molt of a hemimetabolous insect which results in the imago

The first demonstration of a hormonal factor controlling insect development was given in 1922 by Kopeć (89) He removed the cerebral ganglion (brain) from freshly molted last instar larvae of a moth, Lymantria In such animals pupation does not take place unless the brain, which in this case appears to play the role of an endocrine organ, is re-implanted into the abdomen Operations of this kind yield conclusive results only if they are performed before the so-called "critical period," *i e*, a definite period at which, within a given developmental stage, the hormone concentration in the circulation reaches an effective level (p 152)

In the following two decades evidence accumulated supporting the conclusion that hormones are instrumental not only in pupation but also in other phases of insect development Consequently the existence of molting hormones, pupation hormones, and metamorphosis hormones was postulated The data available now have been obtained in representatives of various groups, *i e*, the hemimetabolous Hemiptera and Orthoptera, and the holometabolous Lepidoptera, Hymenoptera, Coleoptera, Megaloptera, and Diptera As may be seen from the following examples these various orders of insects differ somewhat from one another in their developmental physiology

A Observations in Various Groups of Insects

In the tropical bug Rhodnius and in other representatives of the Hemiptera (Triatoma, Cimex) the dorsal region of the protocerebrum (Fig 8) furnishes a "molting hormone" If this part of the brain, taken from donors during the critical period, is implanted into decapitated nymphs, *i e*, nymphs deprived of their own source of hormone, molting results (184) The reaction is specific and cannot be induced by implants of other parts of the central nervous system or of other organs This localization offers strong evidence that the hormone which causes molting originates in the neurosecretory cells of the pars intercerebralis Such cells which combine nervous and glandular characteristics have been demonstrated not only in Rhodnius (70,184) but also in a variety of other insects (p 149)

In addition to this factor Wigglesworth (180,181) postulated the existence of a juvenile (inhibitory) hormone the source of which he

localized on indirect evidence in the corpus allatum (Fig 8a) The juvenile factor restrains adult differentiation for a time sufficient to permit the necessary degree of growth In other words it controls the rate of development Although this view has not been accepted by all workers in the field (112 114 116 117) it is supported increasingly by experimental evidence obtained in various groups of insects (*cf* Section V of the following chapter for a similar phenomenon in crustaceans)

The endocrine role of the corpora allata has been more firmly established in several species of Orthoptera (Fig 6) in which the extirpation and implantation of this gland has been possible

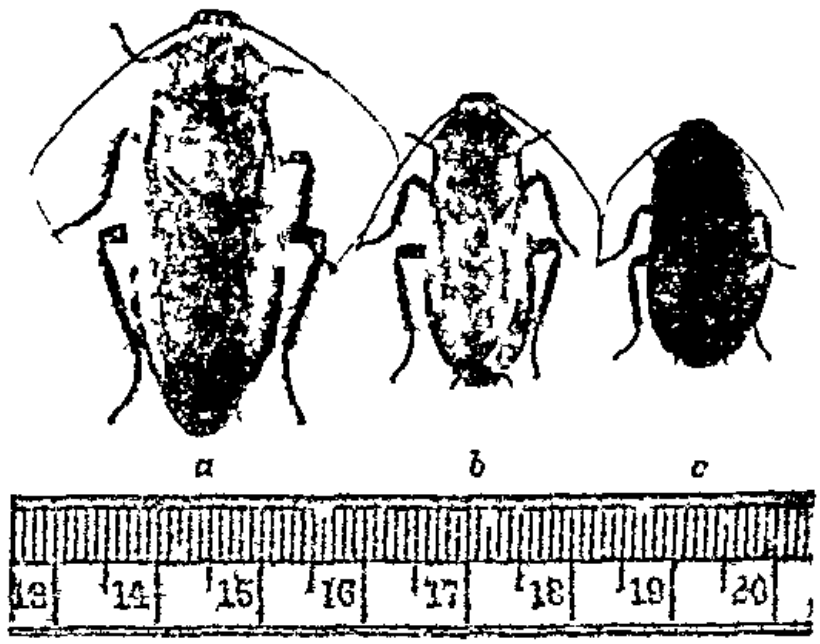


Fig 1 —(a) Normal adult male of *Leucophaea maderae* (b) Adultoid male obtained from allatectomized seventh instar nymph (c) Normal male eighth instar nymph Scale in centimeters (From Scharrer 140 courtesy of Charles C Thomas Publisher Springfield Illinois)

(1) After allatectomy in early nymphal instars of Dixippus (112) Bacillus (53) and Leucophaea (140 Fig 1b) adult differentiation sets in prematurely Molts are suppressed and development is abbreviated

(2) Implantation of corpora allata into normal last instar nymphs retards metamorphosis and is followed by supernumerary molts (110 116 117 Fig 2b) This effect may be obtained even with grafts from adult donors in some insects but not in others

In the first type of experiment the animals resulting after the final molt are smaller in the second type larger than normal adults But difference in size is not the only characteristic that distinguishes these

experimental animals from normal adults In the allatectomized animals, developing precociously because of lack of juvenile hormone, the developing tissues do not seem fully prepared to undergo imaginal differentiation In Leucophaea they are less ready in younger nymphal stages than in older ones Insects that resemble adults to a greater or less degree (adultoids) are the result (Fig 1b) Correspondingly in animals which have been kept overtime in the nymphal condition by allatum implants, the organs ready for imaginal differentiation, are subjected to the prolonged action of the juvenile hormone These animals likewise show a mixture of nymphal and imaginal characters, they may be called nymphoids (Fig 2b)

The role of the corpora allata in the control of development is essentially the same in the holometabolous Lepidoptera Allatectomy in young larvae causes precocious development (17,18,20,22,58,92,121,123, 187) Allatum implants from pupae in diapause into hosts ready to complete adult development do not inhibit this process (187)

The endocrine significance of the brain in bringing about pupation in this group has already been referred to (p 123) The earlier results of Kopeć (89) were later confirmed by several investigators (33,92 119,125) The control of adult development by a substance originating in the brain has recently been demonstrated by Williams (187) in "diapausing' (dormant) moths Platysamia pupae when chilled (2–5°C) for four to six weeks after pupation and subsequently returned to room temperature (25°C) complete their development after an additional four to six weeks By contrast, in pupae left at room temperature diapause lasts at least five months Brains from donors ready to metamorphose implanted into dormant hosts bring about adult development in the latter Brains, up to eight in number, taken from dormant donors do not have the same effect on the host, neither do a variety of other tissues Brainless pupae fail to complete their development, unless the extirpation has been performed after the critical period which is about fourteen days following the return to room temperature Implants of "activated" (nondormant) brains into decerebrated pupae restore the capacity of the host to complete metamorphosis

Of much interest is the observation that the proper effect is obtained only if the brain implants establish "intimate continuity" with tissues of the host (Williams, personal communication[2], see also 8 21) The results after extirpation and implantation of brains agree with those obtained in parabiosis experiments (187)

Localization experiments showed that a specific region of the brain, *i e*, the inner mass of the cerebral lobes, is responsible for the elaboration

[2] I wish to thank Dr C M Williams for permission to use these unpublished data

of the active principle. This region contains two groups of neurosecretory cells, one medial and one lateral. Implants must contain both glandular centers to be effective; parts of brain tissue lacking one or both cell groups are incapable of terminating diapause (187b). This localiza-

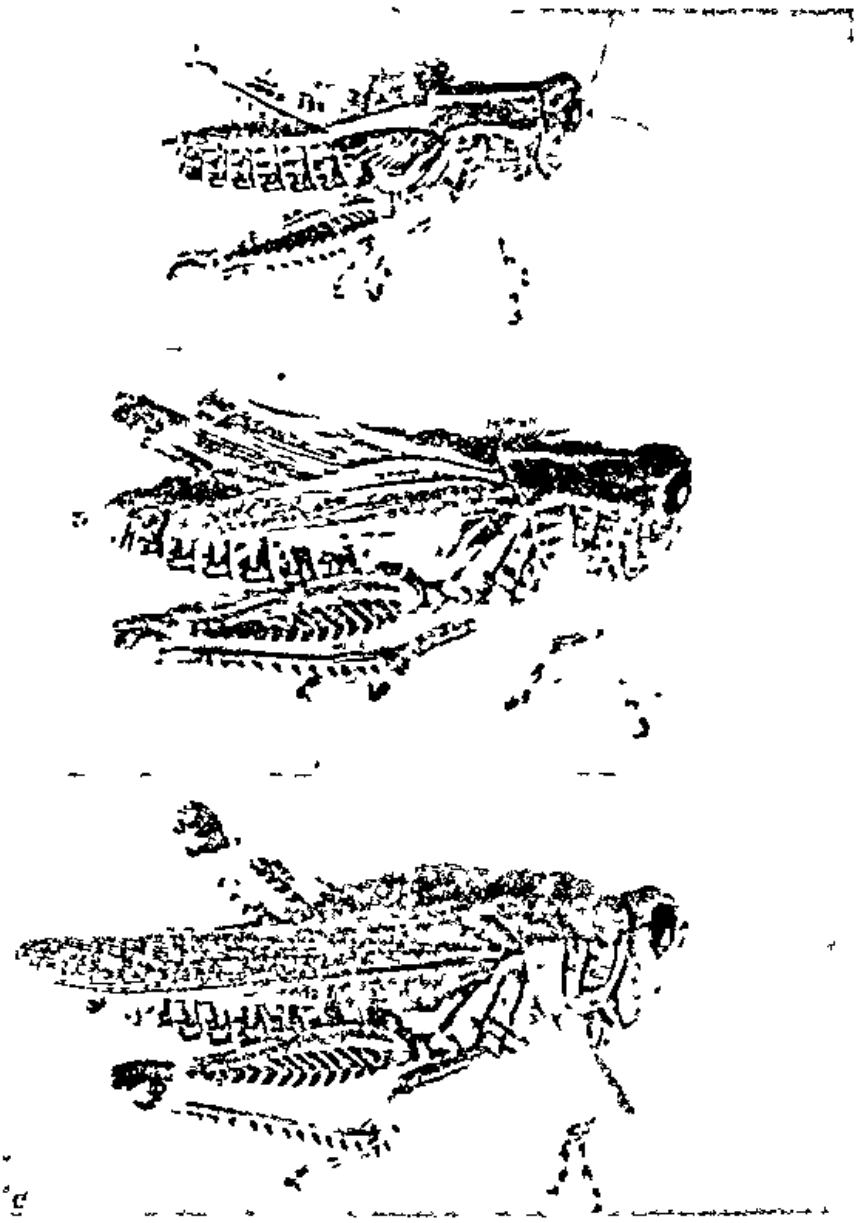


FIG 2.—(a) Normal sixth instar nymph of *Melanoplus differentialis*. (b) Nymphoid female obtained by implantation of corpora allata. (c) Normal adult female (From Pfeiffer, 110)

tion of the physiological effect in the neurosecretory centers of the brain of Platysamia agrees with the results of Wigglesworth in Rhodnius (p. 123).

The brain factor has not been demonstrated in the blood. Implants

of larval brains, after undergoing a sufficient degree of development in the host and after being activated by chilling bring about imaginal development in brainless hosts [3]

In addition to the brain the prothoracic glands are necessary for the completion of adult differentiation Both sources of physiologically active substances must be implanted in order to bring about imaginal changes in a pupal abdomen isolated by means of a ligature Unlike brain grafts, the prothoracic glands from a diapausing donor are as active as those from a nondormant pupa (187) This and other evidence (58) makes it seem quite probable that in the endocrine control of pupation as well as of imaginal differentiation the influence of the brain is superimposed over that of the prothoracic glands

Also in other moths (Bombyx) the endocrine significance of the prothoracic glands for the control of pupation and imaginal differentiation, and apparently also for molting, has been demonstrated (57 58 59, see also 8,19,22a)

Various developmental stages of Platysamia show both qualitative and quantitative differences in metabolism The cytochrome content of diapausing pupae is considerably smaller than that of active stages (pupae shortly before emergence and larvae) These and additional data indicate a causal relationship between endocrine mechanisms and biochemical changes in the developing tissues (187b)

A hormone causing pupation and imaginal differentiation in certain Hymenoptera seems to originate in the brain (146)

In the development of Coleoptera the role of the corpus allatum was found to be the same as in other groups of insects (131)

Ligation experiments made on larvae of Sialis, a representative of the Megaloptera, demonstrate the existence of a center controlling metamorphosis, located in the region of the third thoracic and first abdominal segments (60,61) Corresponding ligatures in pupae are without effect on the completion of metamorphosis (104) If in parabiosis experiments larvae in the beginning phase of metamorphosis are joined with younger specimens, the latter metamorphose prematurely and synchronously with their partners The source of the hormonal substance involved is not known Extirpation and implantation of ganglia located in the critical region have no influence on development

In the muscoid Diptera developmental hormones are furnished by the ring gland (Fig 7), a composite organ containing corpora allata and cardiaca (40,128,143, see also 162a,164a) This gland controls growth and molting, pupation, and adult differentiation

Bodenstein (16) transplanted larval heads together with ring glands

from mature larvae into the abdomen of adult Drosophila The result was that the transplanted heads underwent one or two molts Molting did not occur when the heads were transplanted without the ring glands

Puparium formation, which was found to be greatly retarded or suppressed in lethal larvae of the Drosophila mutant lgl and in certain hybrids, can be induced by the implantation of genetically normal ring glands (66) This action of the ring gland was confirmed in normal larvae of Drosophila (68,165) and of Calliphora (6, see also 43,55) In the latter form extirpation of the ring gland prevents puparium formation (24) Pupation proper which takes place within the puparium likewise is controlled by the ring gland (103,167,167a) Implants of brains without ring glands have no effect on pupation (170) or puparium formation (68,165)

In Calliphora growth of imaginal discs is arrested after removal of the ring gland (24) Similarly the development of organ discs in Drosophila was shown to be under the control of this gland (10,11,14,15,163 164,167,170 172) During an earlier phase of development the ring gland mainly promotes growth of the discs, later their imaginal differentiation This change in response is brought about not only by an increased hormone production as the ring gland matures, but also by the altered responsiveness of the developing tissue (tissue competence) However in younger discs a certain degree of differentiation also takes place (170)

In addition to the imaginal discs the differentiation of other organs of these dipterous larvae, for example the brain (165) and the gonads (p 134), takes place under the influence of the ring gland These changes are correlated with the dedifferentiation of larval structures such as the midgut epithelium and the fat body (167)

The hormone (GD hormone, p 130) bringing about puparium formation and imaginal differentiation in muscoid Diptera originates in the larger gland cells (Fig 7b) which are now known to be corpus cardiacum tissue (see the discussion of the homologies of the ring gland components by Poulson 128) This conclusion is based on histological as well as experimental evidence (a) The deficiency of lethal ring glands as compared with normal ones concerns the large cells (143), (b) Histological signs of secretory activity of the large cells coincide with phases of physiological activity of the gland (163,167,172, see also 169), (c) Implants of ring gland fragments consisting mainly of large gland cells furnish GD hormone to the host (170)

Further observations of interest are that young ring glands as well as older ones furnish the GD hormone, and that this activity apparently follows a cyclic pattern An analysis of this cycle of hormone production

suggests that the GD hormone in Drosophila larvae also controls molting (170) An as yet unexplained observation concerns the effect of adult corpora cardiaca on *Drosophila hydei* larvae Implants of these glands, in contrast to control implants of fat body or corpora allata, cause a marked delay in puparium formation (172a)

B Discussion of Results

It is evident that the information concerning the hormonal factors involved in various phases of insect development is still fragmentary Some of the data reported above may appear divergent If it is assumed that each step in postembryonic development is governed by one (or several) specific hormones, *i e*, molting pupation, and metamorphosis hormones, it becomes difficult to compare the various hormonal factors in one group of insects with those in another Obviously in a hemimetabolous form there would be no need for a pupation hormone as in a holometabolous insect Furthermore, even within the holometabolous group, comparable hormones such as the pupation hormones of a moth and of a fly seem to differ with regard to their source in the body

Therefore it may seem too early or even impossible to establish a common denominator for the data at hand Nevertheless certain fundamental trends are becoming increasingly apparent which justify a preliminary attempt at a more uniform interpretation of the hormonal control of insect development This tentative interpretation is based on concept introduced by Wigglesworth (p 123) and can be formulated in the following manner

In holometabolous as well as hemimetabolous insects each developmental step may be viewed as governed by a balanced interaction between two developmental hormone systems on one side and the growing and differentiating tissues on the other

It has been briefly stated before (p 128) that the developing tissue gradually changes in its capacity to respond to endocrine stimuli Consequently in a given hormonal environment the reaction is determined by the responsiveness of the tissue For instance in the same hormonal environment the type of response, growth or imaginal differentiation, is determined by the age of the imaginal discs (168) Furthermore, at the same stage of development various anlagen, such as salivary gland eye, genital discs even various regions within the same anlage may differ in their response (15)

The two types of hormone collaborating in the control of development are (I) the "growth and differentiation hormone (GD hormone or hormones), (II) the juvenile hormone (inhibitory hormone, corpus allatum hormone)

A hormonal factor of type I (GD hormone) activates the imaginal ("imaginipetal," Vogt, 170) potencies to an extent which is regulated by the responsiveness of the developing tissue It promotes growth and imaginal differentiation of tissues and organs In the immature insect, growth takes place under the influence of a GD hormone in periodic steps as evidenced by the occurrence of successive molts For this reason Wigglesworth (180,181,184) and others called this factor "molting hormone" When this term is used it should be kept in mind that, in addition to bringing about molting, this factor also promotes imaginal differentiation Therefore, the molts it causes are not "simple," *i e*, larval or nymphal molts, but molts coupled with metamorphosis It follows that the "molting hormone" alone cannot account for the occurrence of larval molts

In order to safeguard the proper number of larval molts there exists an additional factor which stimulates the juvenile, *i e*, larval or nymphal potencies of the developing tissues This juvenile (inhibitory) hormone modifies the action of the GD hormone, the combined action of both factors causes larval molts

By keeping the developing insect in the immature stage the juvenile hormone favors (or permits) the occurrence of molts This fact should, however, not be interpreted as an indication that the juvenile factor as such acts as a molting hormone According to experimental evidence the juvenile factor, when acting alone, is incapable of causing a molt On the other hand, molts may occur in the absence of the juvenile hormone as in allatectomized animals For this reason the use of the term "molting hormone" for the juvenile hormone (13) is not recommended Its use would also lead to confusion since this term has been previously employed by Wigglesworth and others with more justification for a different hormone

One of the reasons why the existence of several types of hormones has been postulated where probably only one is necessary was that the GD hormone originates in organs as different as the brain of a caterpillar and the ring gland of a fruit fly larva The following discussion is intended to show that these differences need not be considered as significant

The three main sources of developmental hormones known at present are (a) the glandular corpora allata, (b) the corpora cardiaca, consisting of nervous and glandular elements, (c) the brain, or more precisely the pars intercerebralis of the protocerebrum containing glandlike nerve cells (Fig 8b)

In all insect species suitable for experimental study the corpus allatum has been shown to be the source of the juvenile hormone However, equally specific roles have not been assigned to the two remaining centers

The physiological significance of both the corpus cardiacum and the pars intercerebralis may be better understood if they are not treated as two separate centers of glandular activity However different they may seem at first sight, there exists an unusual morphological relationship between them, as has been demonstrated in the orthopteran *Leucophaea maderae* The corpora cardiaca consisting of nervous and glandular elements are innervated by fiber bundles originating in the pars intercerebralis, a brain center which itself is characterized by the occurrence of secreting nerve cells Furthermore, colloid masses are found along the nerves (*nervi corporis cardiaci*) connecting these two neuroglandular centers It would be difficult to assume that this striking morphological feature is without physiological significance Therefore, on the basis of this relationship, which has a counterpart in the hypothalamo hypophyseal system of the vertebrates (144, see also 76), it has been proposed to consider the pars intercerebralis and the corpora cardiaca as components of one neuroendocrine complex

As to the physiological mechanism of this glandular complex there are two possibilities either both the brain and the corpus cardiacum cooperate in the elaboration of GD factors, or in certain animals the one, in others the other, component has become the predominant hormone source Considering the variability in the development of neuroglandular organs in insects one may expect to find examples of either alternative among the various groups of insects, an expectation which is borne out by data discussed elsewhere (144)

Aside from the intercerebralis-cardiacum allatum system, only the prothoracic glands of certain moths have recently been demonstrated as the source of a factor concerned with development (57,58 59,187) However, this factor, lack of which prevents metamorphosis, appears to be subordinated to or otherwise linked with the GD hormone furnished by the brain

In summary, two types of developmental hormones exist which originate in two types of glands The one type, *i e*, the GD hormone (or hormones) is produced by the neuroglandular intercerebralis-cardiacum complex, the other, *i e*, the juvenile or inhibitory hormone, by the glandular corpus allatum

This "two-hormone concept" can be applied to the great majority of experimental data available at present It has been stated (p 123) that in hemimetabolous insects the more drastic changes leading from the immature to the mature insect occur in the last nymphal instar But imaginal differentiation is not entirely restricted to the last stage It takes place also in a small measure during earlier nymphal life In an attempt to explain the hormonal mechanism it may be postulated that

both the GD hormone and the juvenile hormone are active in all stages except the last In the last stage none or at most only an ineffective amount of juvenile factor is released [2a] Therefore, under the uninhibited stimulus of the GD hormone the final, *i e*, the major, step of metamorphosis can take place in the last stage (180,181,184).

Theoretically two possibilities exist in explanation of the small changes in younger nymphs (a) In consecutive nymphal stages the hormone balance is shifted in favor of the GD hormone by a gradual decrease in the relative amount of juvenile hormone released into the circulation In this case the responsiveness of the developing tissues may be assumed to remain approximately the same in all nymphal stages (b) The ratio of both developmental hormones remains unchanged in each nymphal stage except in the last, but the growing tissues are increasingly capable of response to the stimulus for differentiation

These alternatives can be subjected to an experimental test by a comparison of the effect of allatectomy on various nymphal stages in the same as well as in different insect species In allatectomized nymphs one factor governing development, *i e*, the juvenile hormone, has been removed Therefore, differences in the events following these operations reflect differences in the relative effectiveness of the two remaining factors, *i e* of the GD hormone and of tissue responsiveness Such differences are apparent, for instance, in a comparison of various stages of Leucophaea (140), a species with an average of eight nymphal instars Allatectomized seventh instars become adultoids at the molt following the operation which thus becomes the final molt Allatectomized sixth or fifth instars at their next molt show only an intermediate degree of imaginal differentiation they require one additional ("preadultoid") stage, and consequently undergo one more molt before they too become adultoids Since both younger and older nymphs have been subjected to the influence of the GD hormone alone, without the effect of the juvenile hormone the quantitative differences in response must have been due to differences inherent in the tissues In Leucophaea and related insects the ratio of developmental hormones although it may, need not change appreciably except in the last instar

If in certain other species such as Rhodnius similar changes in tissue responsiveness exist throughout the course of nymphal development, they seem less obvious than in Leucophaea In Rhodnius, according to

[2a] In a recent publication (184a) Wigglesworth suggests that the corpus allatum of last instar nymphs of Rhodnius not only ceases to secrete the juvenile hormone but also actively removes from the blood any traces of the juvenile hormone that remain

Wigglesworth (180), nymphal tissues prove ready for adult differentiation at a very early stage "Diminutive adults" result at the molt following the operation even when first instar nymphs are used Therefore, in Rhodnius a gradual decrease in the activity of the juvenile hormone during nymphal life may account for the small changes observed in successive instars

The conclusion that in the last stage no effective level of juvenile hormone is reached under normal conditions can be substantiated in two ways (a) allatectomy in last instars, regardless of the species studied, has no apparent effect on the course of development (20,108,140 146,175), (b) allatum implants in last instars cause supernumerary molts and prevent the completion of metamorphosis Hence last instars also respond to the action of the juvenile factor, if it is present (20,110,123,140)

Evidence obtained in holometabolous insects likewise suggests that the two factors (GD hormone and juvenile hormone) collaborate during the phase of periodic growth (see also 76a) This larval phase is followed by a phase of adult differentiation Pupation and metamorphosis can be explained as taking place through the action of one (or several) GD factors in the absence of an effective amount of juvenile hormone

This conclusion is demonstrated by the fact that allatectomy and implantation of corpora allata have comparable effects in holometabolous and hemimetabolous insects In Lepidoptera, for example, the period of growth or larval period is prematurely ended when young larvae are allatectomized, and supernumerary molts result when normal last instar larvae receive allatum implants (see above)

The "two-hormone concept" as elaborated in the preceding analysis may or may not apply to insects other than those discussed so far In the highly specialized muscoid Diptera, where corpus allatum and corpus cardiacum are contained in one organ, the ring gland, these sources of developmental hormones cannot be analyzed separately as readily as in other groups The ring gland brings about molting as well as pupation and imaginal differentiation There is good evidence that the GD hormone controlling these processes originates in the large (cardiacum) cells of the ring gland (p 128), and that it is produced throughout the entire larval life in varying quantity (14,170) The precise function of the allatum cells in the development of these insects is still unknown However, there is no indication that the allatum component of the ring gland of fly larvae and pupae acts differently from the corpus allatum of other insects The assumption that it too furnishes a juvenile hormone is based on certain histological as well as experimental evidence (25,169 170,172a)

III. Role of Hormones in Reproduction

In the adult insect a hormone or hormones originating in the corpora allata play an important role in reproduction The existing relationships express themselves in two ways (1) in effects of the corpora allata on the gonads and on the accessory sex glands, (2) in an influence of the gonads on the corpora allata Whereas these relationships are well established, the action of sex hormones has not been demonstrated satisfactorily

A Effect of Corpora Allata on Gonads

Ovaries It has been shown in a variety of insect species that the normal function of the ovaries is under the control of a corpus allatum hormone Wigglesworth (181) demonstrated in the hemipteran *Rhodnius prolixus* that the eggs fail to mature in the absence of the corpus allatum (see also 101a) According to Pfeiffer (108,111,174) in allatectomized females of the orthopteran *Melanoplus differentialis* egg development stops at the beginning of the period of yolk deposition In another orthopteran, *Leucophaea maderae*, the presence of the corpora allata was shown to be necessary throughout the period of growth and yolk deposition, which in this species constitutes about the first third of the total period required for the development of the eggs (141) Re implantation of corpora allata into allatectomized females of these orthopterans restores their capacity to produce mature eggs

A similar hormonal relationship is known to exist in the Diptera Drosophila (160,161,162,171, see also 16a), Calliphora, Musca (155,156, Fig 3), Lucilia, Sarcophaga (41), and Anopheles (47a) By means of transplantations in Drosophila larvae it was found, for example, that ovaries of *D melanogaster* in *D funebris* hosts develop mature eggs only if melanogaster ring glands are grafted together with the ovaries The hormone furnished by the melanogaster ring gland seems to be qualitatively different from that of the funebris ring gland On the other hand grafted ovaries of Calliphora develop in Lucilia and *vice versa* (156) under the influence of the ring gland of the host In Lucilia and Sarcophaga, denervation of the corpus allatum has the same effect on egg development as extirpation (41) In the groups of insects discussed so far, corpus allatum from male as well as female donors may furnish the hormone necessary for the maturation of the eggs (109,156,160,181)

The aquatic beetle Dytiscus (84) has an annual cycle of ovarian activity, with the laying period normally starting in March or April Females can be induced to lay eggs during the winter (resting period) by implantation of five or more pairs of corpora allata The similarity of this effect to that of hypophyseal implants in winter frogs (188) is of

interest At all times of the year allatectomy in Dytiscus prevents egg development and, as in other species studied (41,108,141,181), causes pronounced regression of the ovaries Apparently the corpus allatum hormone makes egg development possible by suppressing the resorption of the oocytes The activity of the corpora allata seems to follow a cyclic pattern, hormone being released about every twelve days

The situation is somewhat different in Dixippus (Orthoptera 112 116), where egg maturation proceeds in allatectomized females unless the extirpation of the glands is performed during an early nymphal stage

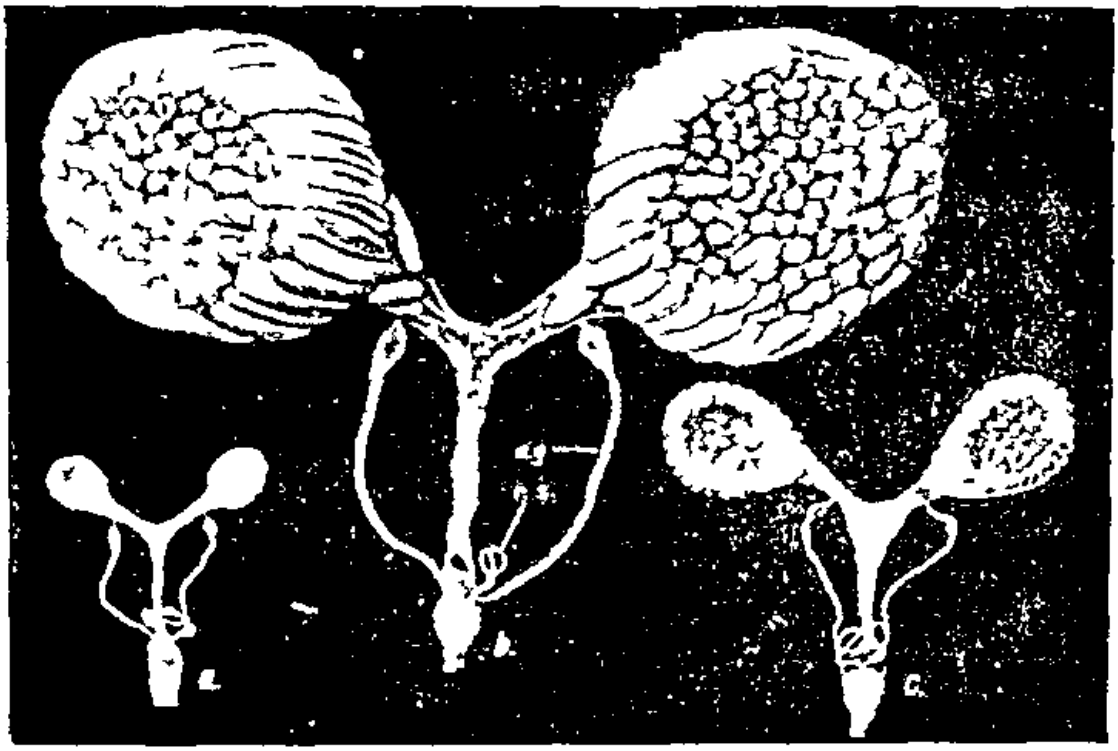


FIG 3 —Reproductive organs of female *Calliphora erythrocephala* (a) Newly emerged fly (b) Mature female (c) Allatectomized female a g accessory sex gland, r s , receptacula seminis (From Thomsen 156)

The presence of the corpora allata is unnecessary for egg production in certain Lepidoptera (18 20,21a,187)

In some orthopterans a possible role of the fat body in egg maturation has been postulated A similar effect has been attributed to extracts of the posterior lobe of the vertebrate pituitary (79) but this finding has not been confirmed (85) [3]

[3] Royal jelly, the special food substance which is supposed to control sexual maturation in the honey bee contains a gonadotropic material Tests made with this material in rats and flies (77 158) were reported to be positive The failure of other investigators (100) to confirm these observations may be due to differences in methods

Testis No relationship between corpus allatum and adult testis has been demonstrated so far Removal of the corpus allatum in males of various species shortly after emergence (41,111,156,181) does not disturb the course of spermatogenesis When mated with virgin females, allatectomized males of the orthopterans Bacillus and Leucophaea are capable of fertilizing the eggs (53,141)

The absence of a noticeable influence of the corpus allatum on the testis in the adult was likewise observed in Drosophila by Vogt (170) Her observation that ring gland implants increase the growth rate and accelerate spermatogenesis in larval and pupal testes is probably attributable to the action of the GD hormone As has been pointed out (p 128) this hormone originates in the large gland cells (cardiacum component) of the ring gland, and implants of this part only have the same effect on the testis as do whole ring glands A similar but less pronounced relationship exists with respect to the pre-imaginal ovary (see also 64)

B Effect of Corpora Allata on Accessory Sex Glands

Regarding the action of the corpora allata on the accessory sex glands the data in the literature are divergent

Females In females of Melanoplus, for instance, the secretory activity of the epithelial lining of the oviduct, which corresponds to the accessory sex glands of other insects, depends on the presence of the corpora allata (108) The same situation exists with respect to the female accessory sex glands in Calliphora (156) Implantation of corpora allata into allatectomized females of Leucophaea restores the capacity of the accessory sex glands to produce normal amounts of secretory material (141 Fig 4) In contrast to the situation in these three insect species, allatectomy has no effect on the female accessory sex glands of Lucilia and Sarcophaga (41)

Males The male accessory sex glands of Lucilia, Sarcophaga (41) and Leucophaea (141) are not affected by allatectomy In Rhodnius (181), however, and to some extent in Calliphora (156) the male accessory glands appear to be under the hormonal control of the corpus allatum

C Effect of Reproductive Organs on Corpora Allata

The relationship in the opposite direction, *i e* the effect of the gonads on the corpora allata has been studied by means of castration In certain insects (Melanoplus, 109, Calliphora, 155, 156, Lucilia 41, see also 166) ovariectomy is followed by hypertrophy of the corpus allatum Females of Sarcophaga (41) and Leucophaea (141), as well as males of Sarcophaga, Lucilia (41) and Leucophaea (141) show no effect on the corpus allatum

attributable to the removal of the gonads or of the accessory sex glands According to Day (41) there is some evidence that, even in those castrates in which the histological appearance of the corpora allata shows no significant change these glands have become "physiologically altered" However, no such change in physiological properties could be observed in Dytiscus (84) Corpora allata from donors which had been ovari-



FIG 4 —Accessory sex glands of female *Leucophaea maderae* (a) Normal control in state of active secretion (b) Glands of female allatectomized shortly after emergence killed six weeks later The glands resemble those of freshly emerged normal female (c) Actively secreting glands after re-implantation of corpora allata into allatectomized female (From Scharrer 141)

ectomized for several months have the same effect on the ovaries of the host as do implants from unoperated donors

D Effect of Gonads on Secondary Sex Characters

The presence of hormones determining secondary sex characters and mating behavior in a manner similar to that known in vertebrates has not been definitely proved or disproved with regard to insects The evidence is at present more against than in favor of the occurrence of sex hormones in this group of invertebrates

The results of experimental castration and of transplantation of gonads obtained by a number of investigators in a variety of species are on the whole negative (see reviews 54,71,88 138) Grafts of gonads of the opposite sex into castrated caterpillars do not alter the secondary sex

characters of the adult moths Surgical castration in larval stages, with one possible exception (129, see also 49,104a), has no influence on the development of the external secondary sex characters or on the sexual behavior of the adult

However, the analysis of cases of "parasitic castration," *i e*, of insects whose gonads are partially or totally destroyed by parasites, is in disagreement with the experimentally obtained results The first case of parasitic castration in an insect was described by Pérez (105) In the bee, Andrena, castration by the parasite Stylops, and consequently referred to as "stylopization," was found to be accompanied by changes in the secondary sex characters A pronounced sexual dimorphism exists with respect to the legs in that only the female possesses a pollen-collecting apparatus (pollen basket) In the infected female this modification becomes reduced to such an extent that the hind legs can hardly be distinguished from those of the male Similarly the color of the clypeus (which is a structural part of the head) changes from black, the normal color of the female, to the characteristic yellow of the male Corresponding changes due to stylopization take place in the male In other insects, for instance in Chironomus (134), similar effects of parasitic castration have been described

E Interpretation of Experimental Data

The general result of the allatectomy and gonadectomy experiments reviewed in the preceding paragraphs is that a relationship exists between corpora allata and reproductive organs in the majority of insect species studied so far This relationship concerns primarily the female, there is only little evidence that the male sexual function depends on the endocrine activity of the corpora allata

There is no doubt that the action of the corpora allata on the reproductive organs is endocrine in character While this general statement holds true, several problems concerning the number of existing allatum hormones and the nature of their action are yet to be solved Thus the question is still undecided whether the corpus allatum hormone controlling the secretory activity of the accessory sex glands is the same as the hormone controlling egg development The fact that nymphoids (p 125) of Melanoplus show oviducal secretion but no yolk production (110) does not necessarily suggest that two different hormones are involved

In ovariectomized females of Bombyx (68a), Melanoplus (108), Calliphora (156) Sarcophaga (41), and Leucophaea (141) the activity of the accessory sex glands is maintained The influence of the corpora allata on these glands must, therefore, be direct and not by way of the

gonads, a fact which does not decide the question of the number of allatum hormones involved

Another problem concerns the possible identity of the gonad-stimulating hormone with the juvenile hormone Pfeiffer (110) discusses the possibility that both actions can be attributed to the same hormone In support of this viewpoint are the findings that in transplantation experiments a hormone acting on the adult ovary is furnished by ring glands of first instar Drosophila larvae (171), and that adult corpora allata of orthopterans may provide juvenile hormone to nymphs (110,117,140, see also 184a) Finally, a factor controlling the secretory activity of the oviducts was found to be present in nymphs of Melanoplus long before their own oviducts begin to secrete (109), an observation which suggests a possible identity of this factor and the juvenile hormone

As concerns the nature of the hormonal action of the corpora allata on the ovaries, two alternatives may be discussed

(a) The corpus allatum produces a "gonadotropic hormone" which, similar to that in vertebrates, acts specifically on the ovary

(b) The influence of the corpus allatum on the developing ova is merely one of the manifestations of a more general function attributable to the corpus allatum hormone

The more specific term "gonadotropic hormone" has been used by various authors (85,156,161) At the same time others (41,115) have expressed the opinion that the various known actions of the corpora allata may be explained by the postulation of a hormone the function of which is the control of certain basic metabolic processes This interpretation is strongly supported by the fact that Pfeiffer (111) recently furnished experimental proof of the existence of a "metabolic hormone" in Melanoplus This hormone, originating in the corpora allata, controls changes in metabolism which are associated with egg development Evidence for the existence of such a hormonal activity was gained in the following way in a series of adult females of varying age, both normal and operated (castration, allatectomy, allatectomy plus castration), the total content of fatty acid, nonfatty dry matter, and water was quantitatively determined

In normal females of Melanoplus, according to Pfeiffer, the early period of adult life, *i e*, several days following emergence, is characterized by a marked increase in the content of both fatty acid and nonfatty dry matter After this period, the end of which marks the beginning of yolk production and of the secretory activity of the oviducts, the metabolic conditions change In normal females no more increase in fatty acid occurs The existing fat stores become reduced until a certain level is reached At the same time the content of nonfatty dry matter and

water continues to rise considerably Removal of the ovaries does not alter these conditions

In contrast, in allatectomized females with ovaries left intact or removed, the rise in fatty acid content continues at a rate comparable to that observed during the early period (see also 41) Nonfatty dry material does not increase in the manner observed in females with their corpora allata intact

These results lead to the conclusion that under the influence of the metabolic hormone of the corpus allatum certain materials necessary for egg development are produced or mobilized, irrespective of the presence or absence of the ovaries

Most of the known data concerning the effect of allatectomy on the course of egg development could be explained on the basis of the metabolic changes brought about by the corpora allata In allatectomized Melanoplus the ova stop their development at about the time yolk deposition begins (108, 111) The effect of allatectomy manifests itself at a similar period in Rhodnius (181), in Drosophila (161), and in Calliphora (156) Furthermore, egg development in Leucophaea (141) depends on the corpora allata up to the time of ovulation, *i e*, throughout the period of growth and yolk deposition

An indication, however, that allatectomy prevents egg development in some way other than by inhibiting yolk formation has recently been furnished by Joly (84) Allatectomized females of Dytiscus, when dissected after a suitable period of time, show complete atrophy of their ovaries "Il s'agit donc, sinon d'une véritable castration, du moins d'un retour à l'état infantile en quelque sorte prépubéral" (Joly, 84, p 131) This result may be interpreted as evidence that, perhaps at least in certain species, the corpora allata furnish a specific gonadotropic hormone in addition to the metabolic hormone More definite information is necessary, however, to establish this point

No conclusion can be drawn at present with regard to the influence that the female gonad exerts on the corpora allata in certain cases Whether this effect is due to the existence of a sex hormone produced by the ovaries or is brought about in some other way remains undetermined

It has been stated previously that the question of the occurrence of *sex hormones in insects in general is still undecided* Convincing as the effects of stylopization on the secondary sex characters may seem at first sight, there is no agreement among investigators as to the interpretation of these data The estrogenic action in vertebrates of materials extracted from insects (82 96 97,149,150) and even from certain kinds of honey (48) offers no proof that in the insect organism these substances have a com

parable function No effect of vertebrate estrogen (folliculin) on insects has been observed (38,83)

It is quite possible that by an approach different from those used in the past the activity of sex hormones in insects may be established This expectation seems justified in view of certain otherwise unexplained phenomena, such as the correlation between flying instinct and maturity of the gonads in certain beetles (189)

IV. Hormones and Color Change

Among invertebrates, crustaceans and insects show pigmentary reactions which are under hormonal control in a manner comparable to that found in certain vertebrates In contrast to the situation in crustaceans (see Section III of the following chapter), color change in insects plays a minor role and is restricted to a few groups Like other animals, insects may exhibit two types of color reactions (a) morphological color change, a slow process consisting in the formation or destruction of pigments, and (b) physiological color change, brought about by pigment migration (expansion and contraction) and thus causing quick changes in appearance

For instance, in the walking stick, *Dixippus morosus*, changes in the color of the background are accompanied by changes in body coloration (1,63,80,130) If Dixippus is kept on a dark background, its skin becomes dark due to pigment expansion This prompt reaction is followed by the slow formation of additional pigment Darkening of the body likewise occurs, irrespective of the color of the background, when the lower halves of the eyes are coated Under normal conditions the coloration of Dixippus shows a diurnal rhythm (176)

The existence of an endocrine rather than a nervous control of this mechanism of color adaptation in insects is demonstrated by the following observations (a) the cells responsible for the color change are not innervated, (b) in skin grafts the coloration changes synchronously with that of the host (81), (c) if in one part of the body the circulation is temporarily interfered with by means of a ligature, the isolated part assumes a pale appearance for as long as the blood supply remains inadequate Absence of hormone in the circulation leads to pigment contraction and the cessation of pigment formation

The exact localization of the hormonal source has so far not been determined The fact that the whole animal becomes pale following the removal of the head (80) indicates that the center of hormone formation must be in the head region Corpora allata and corpora cardiaca may be involved, in spite of the fact that extirpation of neither of these glands

alters pigmentary reactions (1,114) A morphological color reaction resulting in distinct color patterns is observed after denervation of the corpora allata in Dixippus Re-implantation of these glands into allatectomized specimens leads to blackening of the hypodermis in the neighborhood of the implant (70,72,114,116)

Aside from Dixippus, few cases of insect color change due to hormonal action have been studied (see reviews 71,85,88,138, see also 76b)

Extracts of corpora cardiaca of several insect species have a strong chromatophorotropic effect in crustaceans (23,157, see also 72,86) Similar but less pronounced effects have been attributed to extracts of cerebral and frontal ganglia of insects, which, however, have been tested only in crustaceans (23)

V. "Gene Hormones"

In insects certain hereditary characters are known to depend for their development on the action of diffusible substances These substances represent the "intermediate links between the genes controlling their production and the final character" (Ephrussi, 51, p 327) Because of certain hormone-like characteristics the gene controlled substances have been called "gene hormones" (2,3,50,51,52,91,126) For a discussion concerning the advisability of continuing the use of the term "hormone" for the substances dealt with in this chapter, see Ephrussi (52) and Becker (5)

Some of the methods by which the existence of these diffusible substances is ascertained are those used in endocrinological research The active principle may be introduced by mouth, by blood transfusion, by injection of extracts, or by addition to organ anlagen *in vitro* Another widely used method consists in the exchange of grafts between animals that contain, and others that lack, a certain gene

With these methods it has been demonstrated that certain organs of donors, possessing a given gene, release a diffusible substance which in hosts lacking this particular gene may cause the development of a character determined by this gene Thus, for instance, the development of the genetically determined eye pigment of certain insects may be modified by the implantation of organs from a different genotype

The first experimental demonstration of this important mechanism was given in 1933 by Caspari (28) in the mealmoth, *Ephestia kuhniella* In this species the wild race (a^+a^+) has a dark brown eye pigment in the development of which a gene hormone, the (a^+) substance, plays a decisive role A mutant strain (aa), lacking (a^+) substance, develops red eyes If however, certain organs such as the testis from wild type larvae are grafted into the mutant larvae, the latter also develop normal

dark eyes instead of the expected red ones This experiment indicates that the nonautonomous development of eye pigment in the host must be caused by the release of (a^+) substance from the grafts into the circulation of the host It further indicates that the host, although deficient in its genetic constitution with respect to one gene (a) and consequently lacking (a^+) substance, retains its capacity to respond to the (a^+) substance if furnished by a graft from a nondeficient donor

Organs of wild type donors which in addition to the gonads may furnish (a^+) substance to deficient (a) hosts are the eyes, the brain and ventral cord, the fat body, and the hypodermis (47,132)

In the experiments reported so far the effects observed are exerted by the implant on the host The grafting procedure may be reversed When wild type hosts receive grafts of deficient organs, the host exerts an influence on the development of the implant For instance, (a) testis grafts in an (a^+) host assume the phenotype of the wild race, an observation which leads to the conclusion that (a^+) substance must be present in the circulation of the host In a similar fashion it can be shown that, in addition to the color of the adult eye, testis, and brain, that of the larval skin, ocelli, and subesophageal ganglion also develops under the influence of the (a^+) substance (31)

Another extensively studied species is *Drosophila melanogaster*, in which two eye color hormones were shown to exist by Ephrussi and Beadle (51) (1) the (v^+) substance (for the character "vermilion", interchangeable with the (a^+) substance of Ephestia, 127), and (2) the closely related (cn^+) substance (for the character "cinnabar") Both of these substances are released by the eyes and the malpighian tubes of Drosophila, whereas the fat body contains only (v^+) substance

Likewise in Drosophila another gene which controls the size of the eye has been demonstrated to act through the intermediary of a diffusible substance In larvae of the mutant Bar in which the eye size of the imago is reduced, administration of extracts of wild type Drosophila larvae (or Calliphora pupae) causes a considerable increase in the number of facets The gene controlled substance (B^+) which thus causes the development of a phenotype resembling the wild type is not identical with (v^+) substance (33b,33c)

As in Ephestia and Drosophila, so in other types of insects certain characters undergo somatic changes by means of diffusible substances Examples are Habrobracon, a parasitic wasp (179), and Bombyx (87) Extracts acting similarly on the eye color development of Ephestia and Drosophila as do the (a^+), (v^+), and (cn^+) substances, can be prepared from a variety of insect species (51,94) However, the substances furnished by these insects do not necessarily have the same effect in the donor

as they do in the host. In *Ptychopoda seriata*, for instance, a mutant (dec) exists whose eye color is light yellow instead of the blackish brown of the wild type (dec$^+$). Implantation of (dec$^+$) gonads into (dec) larvae has no influence on eye pigmentation; yet both (dec$^+$) and (dec) grafts furnish (a$^+$) substance when tested in (a) Ephestia (148). Evidently the donor itself cannot utilize the (a$^+$) substance This result in Ptychopoda suggests that gene-controlled reactions other than those resulting in (a$^+$, v$^+$, cn$^+$) substance may be involved in the process of eye pigment formation Actually, in Drosophila the intervention of two genes, (cd$^+$,

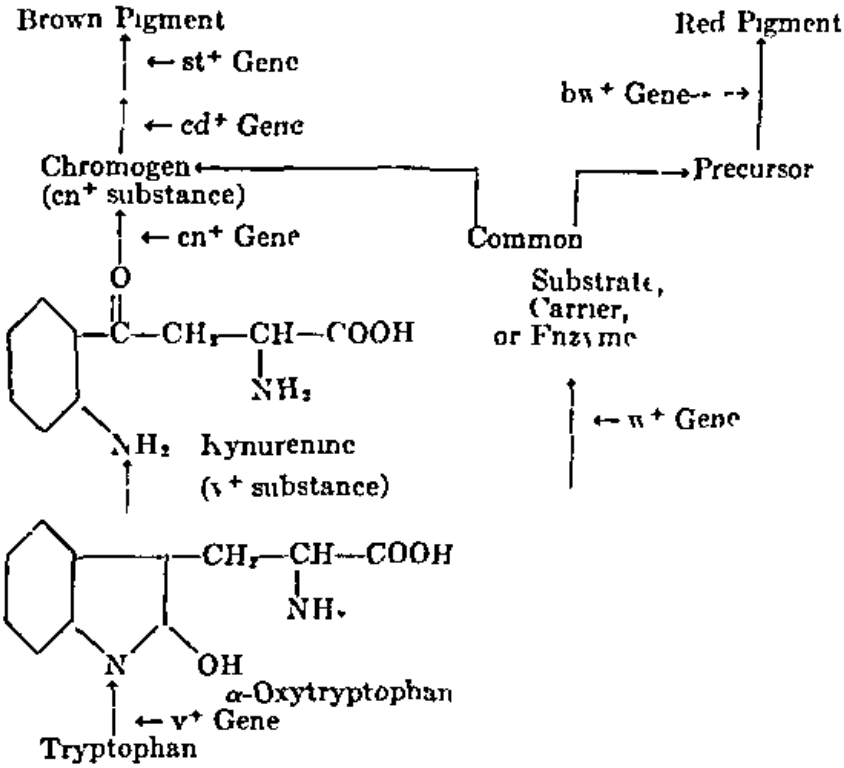


FIG 5 —Scheme of development of eye pigments in Drosophila Vertical arrows indicate steps in the reaction chain, horizontal arrows indicate the places where normal (wild type) genes are necessary for the next step of the reaction (From Beadle, 2)

"cardinal") and (st$^+$, "scarlet"), in addition to (v$^+$) gene and (cn$^+$) gene, is necessary for the formation of the brown pigment (Fig 5).

In recent years a series of investigations reported in rapid succession led to the determination of the chemical nature of the eye color hormones (see 51,52) First an analysis of the chemical properties of purified extracts suggested that the eye color hormones resemble amino acids Feeding experiments then established tryptophan as a most likely precursor of (v$^+$) substance. Tatum and Beadle (153) succeeded in crystallizing a material having the physiological effects of (v$^+$) substance, which they had obtained from bacterial synthesis (151) The (v$^+$, a$^+$)

substance could finally be identified as kynurenine, a derivative of tryptophan, by these means (1) Butenandt, Weidel, and Becker (26) showed that L-kynurenine has the same physiological and chemical properties as (a^+, v^+) substance, while D kynurenine as well as kynurenic acid cannot replace either the (v^+) or the (cn^+) substances, (2) the active principle synthesized from L-tryptophan by certain bacteria was identified as a sucrose ester of L-kynurenine, the L-kynurenine being the active portion of the molecule (154), (3) kynurenine was demonstrated to occur in Drosophila pupae and Bombyx eggs (87)

In the insect organism kynurenine is apparently formed by way of 2-hydroxytryptophan (α-oxytryptophan, prokynurenine) from tryptophan This chain of reactions appears to take place by means of an enzymic system which is activated by the (v^+, a^+) gene (27,87) Similarly the next step, from kynurenine to (cn^+) substance, depends on the action of the (cn^+) gene *In vitro* experiments show that the pigmentation of explanted Drosophila eyes in a medium containing kynurenine may be inhibited by the addition of KCN (37) In the mutant strains the enzymic oxidation of tryptophan may be inhibited, an assumption which is supported by the finding that (aa) Ephestia contains significantly more tryptophan than (a^+a^+) Ephestia (30,32a,32b) An alternative would be that in (aa) Ephestia less tryptophan is available due to the synthesis of qualitatively different proteins in this strain (32)

The most recently discussed questions concern the nature of the (cn^+) substance, and the mechanism by which gene-controlled substances influence the development of eye pigments There is good evidence to support the assumption that the eye color hormones are chemical precursors of certain eye pigments (32b) A quantitative study in Ephestia (90) showed that the amount of eye pigment formed is directly proportional to the amount of kynurenine administered The hypothesis that the (cn^+) substance which is derived from kynurenine represents the chromogen of the brown eye pigment of Drosophila (an "ommochrome", 5) is based on the finding (87) that Drosophila strains containing (cn^+) substance yield a positive Ehrlich diazo reaction The conclusion that it is the (cn^+) substance itself which brings about the positive reaction is *suggestive, although it has not been definitely proved*

Accordingly, the development of one of the two independent eye pigments of Drosophila, the brown pigment, may be visualized to take place as indicated in Fig 5

The mechanism by which the red eye pigment of Drosophila develops is as yet little understood, it is known, however, that its development does not depend upon the presence of diffusible tryptophan derivatives There exists a common step in the development of the brown and red

pigments, but the reaction chains leading to the formation of these pigments are different

VI Sources of Insect Hormones

The organs in the insect body which are either known or assumed to be sources of hormones are summarized in Table I Among these the corpus allatum is perhaps the most versatile gland of internal secretion in this group of invertebrates Its action in developmental and reproductive processes as well as color adaptation has been discussed (see Sections I, II, III) Aside from these functions a certain influence of the corpora allata on tissue growth and maintenance has been demonstrated (a) allatum implants in adult Dixippus restore the capacity to regenerate lost extremities (115), (b) degenerative processes or uncontrolled tumor like growth may take place in Dixippus nymphs after allatectomy in certain parts of the body (musculature, malpighian vessels, fat body, nervous system, 114),[4] (c) after removal or denervation of corpora allata in newly emerged adult flies the imaginal fat body and the oenocytes show signs of regression while the larval fat body fails to disappear completely (41) Furthermore, there exists in Melanoplus a possible correlation between corpus allatum hormone and blood color (110) It is possible that all these actions are correlated with the regulative effect which the corpora allata appear to have on metabolic processes

Implantation of corpora allata into normal last instar nymphs causes regression of the corpora allata of the host (116)

The number of existing allatum hormones is not known (p 139) Certain investigators maintain that one hormone may account for all the various effects attributed to the corpus allatum

The corpus allatum (Fig 6) is a glandular organ whose morphological relationships have been studied extensively (33a,46,73,75,75a,102,103a) It is situated in the head or anterior thorax and is paired in most insects but unpaired in some, such as Rhodnius (Fig 8) Histological signs of secretory activity (cytoplasmic inclusions, acidophilia, vacuoles, lobated nuclei) are more pronounced in some insect species than in others (42,62, 112,147,169) The increase in size of the corpus allatum during the adult stage (47a 78 156) and the sometimes pronounced sexual dimorphism (75,113 147) seem to be manifestations of differences in physiological activity

[4] It may be remarked that in this case tumor like growths appear actually to be caused by an endocrine disturbance, inasmuch as their occurrence can be prevented by corpus allatum implants Tumors in various organs were also observed in a different species (*Leucophaea maderae*) after allatectomy, but their origin has definitely been traced to the incidental cutting of the recurrent nerve (139)

TABLE I

Endocrine organ	Physiological process—type of hormone	Orders of insects	Authors
Corpus allatum	Development—juvenile (inhibitory) hormone	Hemiptera	Wigglesworth 1934–47
	Reproduction—gonadotropic hormone?	Orthoptera	Pfeiffer 1936–45, Pflugfelder 1937–41, Scharrer 1946
	Reproduction—metabolic hormone	Lepidoptera	Kühn and Piepho 1936–42, Bounhiol 1936–44
	Metabolism—metabolic hormone	Coleoptera	Radtke 1942, Joly 1945
	Color change?—type of hormone undetermined	Diptera	E Thomsen 1940–42, Day 1943, *et al*
Corpus cardiacum	Development—GD hormone?	Orthoptera	*Pfeiffer 1939*
	Color change?—(chromatophorotropic hormone acting on crustaceans)		Brown and Meglitsch 1940, M Thomsen 1943
Ring gland	Development—GD hormone, juvenile hormone?	Muscoid Diptera (larvae & pupae)	Hadorn 1937–41, Burtt 1938
	Reproduction—gonadotropic hormone?		Bodenstein 1938–44, Vogt 1940–44, *et al.*
Brain	Development—GD hormone	Lepidoptera	Kopeć 1922, Kühn *et al* 1935–38, Williams 1946
		Hemiptera	Wigglesworth 1940
		Hymenoptera	Schmieder 1942
	Nonautonomous development of hereditary characters—*"gene hormones"*	Lepidoptera, Diptera	Ranzi 1939
Prothoracic glands	Development—hormone probably acting in cooperation with GD hormone of brain	Lepidoptera	Fukuda 1940–41, Williams 1946

TABLE I (*Continued*)

Endocrine organ	Physiological process—type of hormone	Orders of insects	Authors
Ventral glands	Development?	Orthoptera	Pflugfelder 1938–39
Pericardial glands	Development?	Orthoptera	Pflugfelder 1938–39
"Corpus luteum"	Reproduction—hormone influencing ovarial cycle?	Orthoptera	Iwanoff and Mestscherskaja 1935
Fat body	Reproduction—gonadotropic hormone?	Orthoptera	Iwanoff and Mestscherskaja 1935, Petrovskaja 1941
	Nonautonomous development of hereditary characters—' gene hormones"	Lepidoptera, Diptera	Beadle, see Ephrussi 1942
Gonads	Development of secondary sex characters—sex hormones?	Hymenoptera, etc	Pérez *et al*, Prell 1915
	Nonautonomous development of hereditary characters—' gene hormones"	Lepidoptera, Diptera	Caspari 1933, *et al*
Eyes, hypodermis	Nonautonomous development of hereditary characters— "gene hormones"	Lepidoptera, Diptera	Clancy 1940, De Mello 1940, *et al*

Like the corpus allatum the neuroglandular corpus cardiacum (Fig 6) was first considered as an endocrine organ on the basis of its histological appearance (33a,45 46,74,113) The amount of secretory material which it contains may be considerable However, the precise role of the corpus cardiacum in the endocrine system of insects is still less understood than that of the corpus allatum

The pronounced chromatophorotropic effect in crustaceans of cardiacum extracts from insects (23,157) demonstrates the presence of a physiologically active substance in this gland (see Section III, C, 5 of the following chapter) However, this experiment does not elucidate the role of the corpus cardiacum in the insect organism

Total or partial extirpation of the corpora cardiaca simultaneously with that of the corpora allata does not alter to any noticeable degree the effects observed after allatectomy alone (41,112,142 156) Cardiacectomy in Dytiscus (84) causes atrophy of the corpora allata and, therefore, the ensuing changes in the ovaries which are comparable to those after allatectomy may well be considered as indirect effects In Melanoplus molting is delayed but not entirely prevented by cardiacectomy (108), a result which cannot be fully explained at present

The most conclusive results concerning the physiological significance of the corpus cardiacum were obtained in muscoid Diptera, in the immature stages of which the corpus cardiacum is represented by the large cells (Fig 7) of the ring gland (40,128) The large cells when tested separately from the rest of the ring gland components (170) produce one or several hormonal factors controlling molting and imaginal differentiation (GD hormone) and ovarial development (gonadotropic hormone ?) Adult corpora cardiaca when implanted into *Drosophila hydei* larvae of a certain age (2 days, 21 hours) cause a delay in puparium formation and a change in the coloration of the puparium (172a)

The corpus cardiacum of certain insects exhibits a peculiar intimate relationship with the brain (p 131) which may explain the inconsistency of some of the results obtained by investigators using different species

With regard to the endocrine activity of the brain there are indications that the active principle reaches the effector organs by tissue continuity rather than by way of the circulation (p 125)

The most likely source of the hormonal substances produced by the central nervous system are the neurosecretory cells (Fig 8, p 123) which have been demonstrated in a variety of insect species (39,70,106,136,137, 144,145,165,177,184)

Considerably less information is available on the endocrine significance of the rest of the insect organs listed in Table I The prothoracic

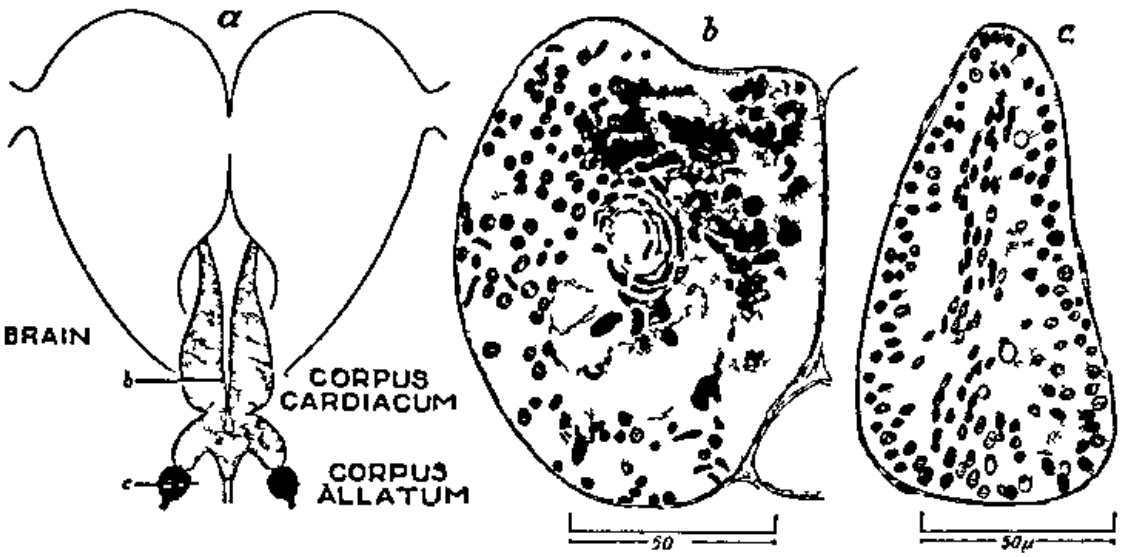


FIG 6 —*Leucophaea maderae* (a) Topography of corpora cardiaca and allata (b) Section through left corpus cardiacum at level indicated in (a) Colloid shown in black (c) Section through corpus allatum at level indicated in (a)

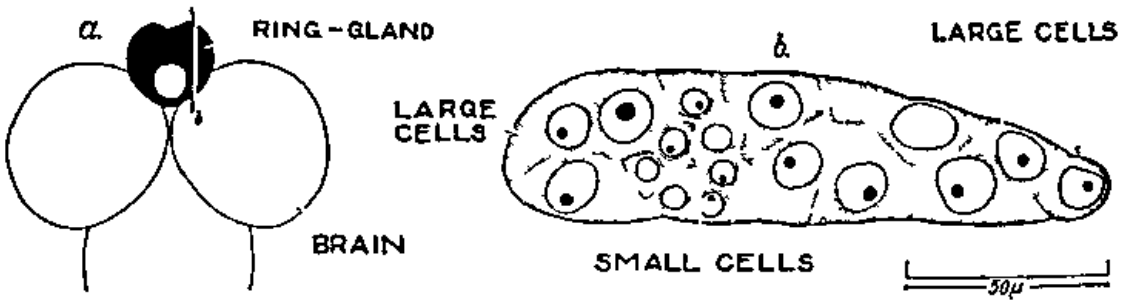


FIG 7 —*Drosophila melanogaster* (a) Topography of larval ring gland (Redrawn from Hadorn 66) (b) Section through ring gland in plane indicated in (a) (Redrawn from Scharrer and Hadorn 143)

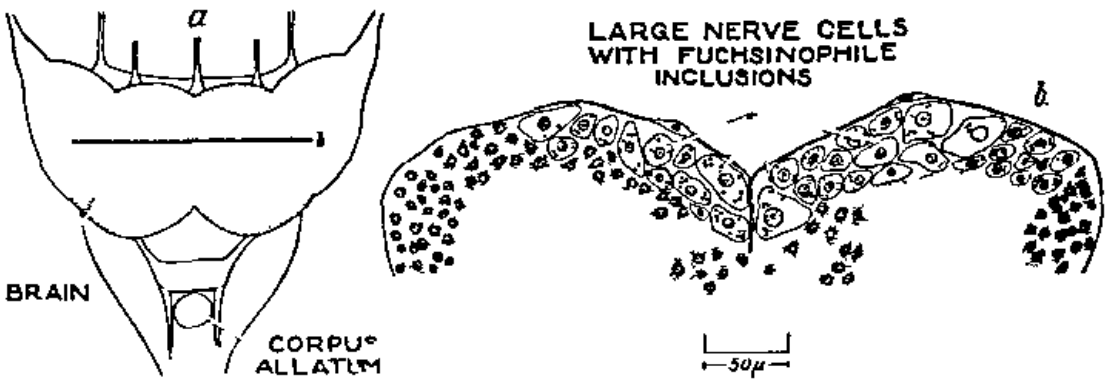


FIG 8 *Rhodnius prolixus* (a) Brain of fifth stage nymph Region furnishing active principle stippled (b) Section through active portion at level indicated in (a) (Redrawn from Wigglesworth 184)

glands (94a,187a) may or may not be active in insects other than the moths discussed above (p 127)

The assumption that the so-called ventral and pericardial glands of Dixippus are of endocrine nature is based on indirect evidence only (114,116) Impressive changes in the histological appearance of these glands are observed after the implantation of young corpora allata into last instar nymphs The pericardial and ventral glands of the hosts not only fail to regress at the expected time, but become considerably enlarged Their nuclei increase in number and size, and become lobated, the cytoplasm shows secretory inclusions Correspondingly, allatectomy in nymphs causes premature degeneration of both ventral and pericardial glands The interpretation of these observations as signs of an intimately related system of endocrine organs seems justified

Aside from furnishing "gene hormones" the fat body is said to be the source of a hormone which brings about maturation of the ovary (79, see also 107) An additional hormone, originating in the area of the "corpora lutea" of the insect ovary has been claimed to keep the ovaries in an immature state for as long a time as the female carries an ootheca (79)

The question as to whether the insect gonads produce sex hormones is still undecided (see Section III, D) Like several other organs listed in Table I, they are a source of "gene hormones"

In addition to the organs discussed the perineurium may be mentioned as a possible endocrine organ (150a), a view which is supported by certain findings in crustaceans

VII Mode of Action and Physicochemical Properties of Insect Hormones

Insect hormones are not specific with respect to the genus or even to the order (6,14,16,51,68,94,120 157,181,187) This statement does not apply to the ring gland hormone of Drosophila which controls the maturation of the ovary (161,164,166, see also 133) The "relative" species specificity of this hormone is comparable to that described for gonadotropic factors in vertebrates (36)

Insect hormones act in very small quantities, their effect depends in some measure on *their concentration* *Low hormone concentrations may* yield partial effects, such as incomplete pupation and metamorphosis (6,8,21b,22b,51,52 61,68,92,93,119,123,181) The effect of a given hormone dose depends on the responsiveness of the reacting tissue (7,12,14, 15,16,68,122,125,168) Once the proper stimulation by a hormone has taken place, as in the case of adult differentiation, the reaction may proceed and be completed during the subsequent absence of the hormone (8,186) On the other hand the responding organs show a considerable

degree of adaptability under varying experimental conditions In experiments in which tissues or organs are grafted into hosts the developmental stage of which differs from that of the donors, synchronous development of host and implants takes place in spite of the difference in age (13,56,67, 68,99,104,109,120,161) In certain organs the determination of imaginal characters occurs later than in others, in some even a reversal to the immature (nymphal) stage is possible (184)

During each developmental stage the hormones controlling postembryonic development appear to be released gradually into the circulation In a given intermolt period the time at which an effective hormone concentration is reached is called the "critical period" (33,89,92,103,125, 184,187) In Rhodnius the critical period of the GD hormone (molting hormone, 180) was found to precede that of the juvenile hormone This result is in agreement with the observation that in Leucophaea the critical period of the juvenile hormone also occurs comparatively late, *i e*, near the beginning of the second half of the intermolt period (140) A possible route by which these hormones are removed from the circulation has been demonstrated in Bombyx where ligation of the malpighian tubes near the intestine may prevent pupation (22b)

The hormonal regulation of certain physiological processes in insects may be influenced by a variety of environmental factors such as temperature, nutrition, humidity (6a,13,29,101,185,186)

In vertebrates and invertebrates alike, little is known regarding the way in which hormones act on cells and tissues One approach to this fundamentally important problem may be offered by the study of the local rather than the general systemic effects of hormones A few interesting observations along these lines have been reported in insects (a) corpus allatum implants in Rhodnius cause more pronounced changes in the skin lying immediately above the site of the graft than in regions farther distant (184, see also 147,172a), (b) in Dixippus the hypodermis in the neighborhood of corpus allatum implants responds with a distinct color reaction (114,116), (c) another localized effect was observed by Joly (84) who implanted into the ovary of Dytiscus corpora allata in numbers insufficient to elicit full ovarial response had they been implanted into the body cavity The oocytes in direct contact with the grafts underwent complete development while the rest of the oöcytes of the same as well as of the other ovary showed incomplete response (Fig 9) This interesting observation requires, however, confirmation based on adequate controls

Thus it appears that endocrine organs of insects are capable of eliciting direct responses on contact with certain tissues Such effects may come about by the interaction of hormones with enzyme systems either

on the cell surface or within the cell Wigglesworth (184) visualizes in the hypodermal cells of Rhodnius two such systems, one imaginal and one nymphal, each being activated by the corresponding developmental hormone

The properties of "gene hormones" have been studied from various points of view These substances are furnished by different organs at different times and in different amounts, they are stored in the organs of formation (34,52) Nutrition is known to influence the production of these substances (152) Their action manifests itself at definite times during development ("sensitive ' or "effective" periods, 33b,33c), but the rate of development does not influence, for example, the output of (a^+) substance (31) The release of "gene hormones" is controlled by

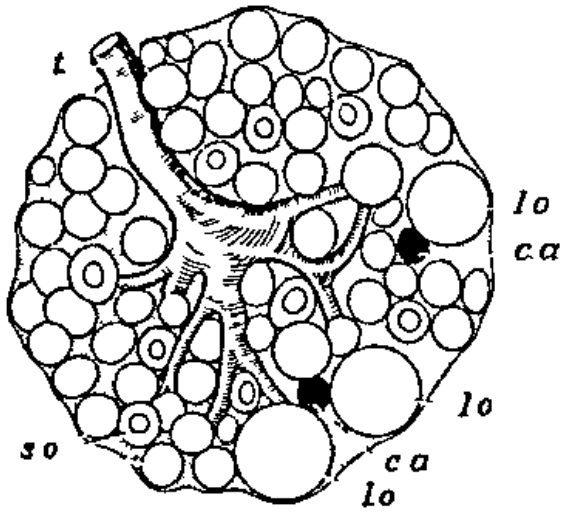


FIG 9 —Intraovarial implantation of corpora allata in Dytiscus c a , corpus allatum implants l o , large oocytes adjacent to the implants s o , small oocytes t , trachea (Redrawn from Joly 84)

the requirements of the organs which produce them ("priority effect") Transmission by the mother to the F_1 generation has been demonstrated The active material acts also *in vitro,* when added to a medium it causes organ anlagen to develop pigment as it would *in vivo* (65) Gene controlled substances participate in the reactions which they bring about, yet they act in exceedingly small quantities For instance, the injection of only 0 012 γ of bacterially produced, crystalline kynurenine has a marked effect on the formation of brown eye pigment (52)

By means of differential extraction with butanol "gene hormones" may be separated from hormones controlling insect development (6)

The hormone causing metamorphosis in dipterous insects (GD hormone) has been purified to the extent that some of its physicochemical properties may be listed as follows the hormone is soluble in water, ethyl

alcohol, acetone, butanol, etc , it is resistant to heat and acids, but very unstable in alkaline solutions, it is dialyzable (4,6) Similarly the chromatophorotropic substance of the corpus cardiacum is known to be soluble in water and alcohol, resistant to heat and desiccation (157) Freezing and drying inactivates the GD hormone of the moth brain (187) and the "gonadotropic hormone" of the corpus allatum of Dytiscus (85) The chemical constitution of these and other insect hormones is unknown with the exception of the "gene hormones" As has been stated before (p 144f) the (a^+,v^+) substance is considered identical with kynurenine, a tryptophan derivative, while the (cn^+) substance is chemically closely related to kynurenine

REFERENCES

1 Atzler, M *Z vergleich Physiol* **13**, 505 (1930)
2 Beadle G W *Chem Revs* **37**, 15 (1945)
3 Beadle G W , and Tatum, E L *Am Naturalist* **75**, 107 (1941)
4 Becker, E *Biol Zentr* **61**, 360 (1941)
5 Becker, E *Z ind Abst Vererb Lehre* **80**, 157 (1942)
6 Becker E , and Plagge E *Biol Zentr* **59**, 326 (1939)
6a Bertani, G *Nature* **159**, 309 (1947)
7 Bodenstein D *Arch Entwicklungsmech Organ* **137**, 474 (1938)
8 Bodenstein, D *ibid* **137**, 636 (1938)
9 Bodenstein D *Biol Zentr* **58**, 329 (1938)
10 Bodenstein D *Genetics* **24** 494 (1939)
11 Bodenstein D *J Exptl Zoöl* **84**, 23 (1940)
12 Bodenstein D *ibid* **86**, 87 (1941)
13 Bodenstein D *Cold Spring Harbor Symposia Quant Biol* **10**, 17 (1942)
14 Bodenstein D *Biol Bull* **84**, 13 (1943)
15 Bodenstein D *ibid* **84**, 34 (1943)
16 Bodenstein D *ibid* **86**, 113 (1944)
16a Bodenstein D *J Exptl Zool* **104**, 101 (1947)
17 Bounhiol J J *Compt rend* **203**, 388 (1936)
18 Bounhiol J J *Compt rend soc biol* **126**, 1189 (1937)
19 Bounhiol J J *Bull biol France Belg Suppl* **24**, 1 (1938)
20 Bounhiol J J *Arch zool exptl gén* **81**, 54 (1939)
21 Bounhiol, J J *Rev zool agr* **40**, 17 (1941)
21a Bounhiol J J *Compt rend* **215** 334 (1942)
21b Bounhiol J J *ibid* **217**, 203 (1943)
22 Bounhiol J J *Compt rend soc biol* **138**, 418 (1944)
22a Bounhiol J J *ibid* **139**, 842 (1945)
22b Bounhiol J J *Compt rend* **220**, 64 (1945)
23 Brown F A Jr and Meghtsch, A *Biol Bull* **79**, 409 (1940)
24 Burtt E T *Proc Roy Soc London* **B126**, 210 (1938)
25 Burtt E T quoted from Wigglesworth (184)
26 Butenandt A , Weidel W , and Becker E *Naturwissenschaften* **28** 63 (1940)
27 Butenandt A Weidel W , and Becker E *ibid* **28**, 447 (1940)
28 Caspari E *Arch Entwicklungsmech Organ* **130** 353 (1933)
29 Caspari E *J Exptl Zool* **86**, 321 (1941)

30 Caspari, E *Science* **98**, 478 (1943)
31 Caspari, E *J Exptl Zoöl* **94**, 241 (1943)
32 Caspari, E *Anat Record* **89**, 545 (1944)
32a Caspari, E *Genetics* **31**, 454 (1946)
32b Caspari, E *Nature* **158**, 555 (1946)
33 Caspari, E , and Plagge, E *Naturwissenschaften* **23**, 751 (1935)
33a Cazal, P , and Guerrier, Y *Arch zool exptl et gén* **84**, 303 (1946)
33b Chevais, S *Bull biol France Belg* **77**, 295 (1943)
33c Chevais, S *ibid* **78**, 1 (1944)
34 Clancy, E B *Biol Bull* **78**, 217 (1940)
35 Craig, R., and Hoskins, W M *Ann Rev Biochem* **9**, 617 (1940)
36 Creaser, C W , and Gorbman, A *Quart Rev Biol* **14**, 311 (1939)
37 Danneel, R *Biol Zentr* **61**, 388 (1941)
38 Dantchakoff, V , and Vachkovitchuté A *Compt rend soc biol* **121**, 755 (1936)
39 Day, M F *Nature* **145**, 264 (1940)
40 Day, M F *Ann Entomol Soc Am* **36**, 1 (1943)
41 Day M F *Biol Bull* **84**, 127 (1943)
42 Day, M F *Psyche* **50**, 1 (1943)
43 De Bach, P *Ann Entomol Soc Am* **32**, 743 (1939)
44 De Beaumont, J *Mitt Schweiz Entomol Ges* **18**, 49 (1940)
45 De Lerma, B *Atti accad Lincei Rend Classe Sci fis mat nat* **17**, 1105 (1933)
46 De Lerma, B *Arch zool ital* **24**, 339 (1937)
47. De Mello, F *Biol Zentr* **60**, 174, (1940)
47a Detinova, T S *Zoologitsheskiy J* **24**, 297 (1945)
48 Dingemanse, I *Acta Brevia Neerland Physiol Pharmacol Microbiol* **8**, 55 (1938)
49 Dobzhansky, I *Arch Entwicklungsmech Organ* **123**, 719 (1931)
50 Ephrussi, B *Am Naturalist* **72**, 5 (1938)
51 Ephrussi, B *Quart Rev Biol* **17**, 327 (1942)
52 Ephrussi, B *Cold Spring Harbor Symposia Quant. Biol* **10**, 40 (1942)
53 Favrelle, M *Compt rend* **216**, 215 (1943)
54 Fleischmann, W Vergleichende Physiologie der inneren Sekretion Perles, Vienna, 1937
55 Fraenkel, G *Proc Roy Soc London* **B118**, 1 (1935)
56 Fukuda, S *Proc Imp Acad Tokyo* **16**, 19 (1940)
57 Fukuda, S *ibid* **16**, 414 (1940)
58 Fukuda, S *ibid* **16**, 417 (1940)
59 Fukuda, S *Annot Zool Japan* **20**, 9 (1941)
60 Geigy, R , and Ochsé W *Rev suisse zool* **47**, 193 (1940)
61 Geigy, R , and Ochsé, W *ibid* **47**, 225 (1940)
62 Gerould, J H *Acta Zool* **19**, 297 (1938)
63 Giersberg H *Z vergleich Physiol* **7**, 657 (1928)
64 Gloor, H *Rev suisse zool* **50**, 339 (1943)
65 Gottschewski, G , and Fischer, I *Naturwissenschaften* **27**, 584 (1939)
66 Hadorn, E *Proc Natl Acad Sci U S* **23**, 478 (1937)
67 Hadorn, E *Rev suisse zool* **48**, 495 (1941)
68 Hadorn, E , and Neel, J , *Arch Entwicklungsmech Organ* **138**, 281 (1938)
68a Hamasaki S *Proc Imp Acad Tokyo* **8**, 267 (1932)
69 Hanstrom, B *Ergeb Biol* **14**, 143 (1937)
70 Hanström, B *Lunds Univ Årsskrift N F Avd 2*, **34**, (16) 1–17 (1938)

71 Hanstrom, B Hormones in Invertebrates Univ Press, Oxford, 1939
72 Hanström, B *Lunds Univ Årsskrift N F Avd 2*, 36, (12), 1–20, (1940)
73 Hanström, B *Kgl Svenska Vetenskapsakad Handl* 18, (8), 1–265, (1940)
74 Hanström, B *Lunds Univ Årsskrift N F Avd 2*, 37, (4), 1–19 (1941)
75 Hanström, B *Biol Generalis* 15, 485 (1942)
75a Hanstrom, B Three principal increterory organs in the animal kingdom *Munksgaard, Copenhagen, 1947*
76 Hanstrom, B *Lunds Univ Årsskrift N F Avd 2*, 38, (8), 1–12, (1943)
76a Henson, H *Biol Rev Cambridge Phil Soc* 21, 1 (1946)
76b Hertz, M , and Imms, A D *Proc Roy Soc London* B122, 281 (1937)
77 Heyl, H L *Science* 89, 540 (1939)
78 Ito, H *Bull Imp Sericult Expt Sta Japan* 1, 63 (1918)
79 Iwanoff, P P , and Mestscherskaja, K A *Zool Jahrb Physiol* 55, 281 (1935)
80 Janda, V *Mém soc sci Bohème*, Cl sci , pp 1–31 (1934)
81 Janda, V *Zool Anzeiger* 115, 177 (1936)
82 Joly, P *Compt rend soc biol* 134, 408 (1940)
83 Joly, P *Compt rend* 214, 133 (1942)
84 Joly P *Arch zool exptl gén* 84, 49 (1945)
85 Joly, P *Ann biol* 21, 1 (1945)
86 Kalmus, H *Z vergleich Physiol* 25, 494 (1938)
87 Kikkawa, H *Genetics* 26, 587 (1941)
88 Koller, G Hormone bei wirbellosen Tieren Probleme Biol Vol I, Akadem Verlagsgesellschaft, Leipzig, 1938
89 Kopeć, S *Biol Bull* 42, 323 (1922)
90 Kuhn, A , and Becker, E Quoted from Becker (5)
91 Kuhn A , Caspari E , and Plagge E *Nachr Ges Wiss Göttingen Biol* 2, 1 (1935)
92 Kuhn, A , and Piepho, H *ibid* 2, 141 (1936)
93 Kühn, A , and Piepho, H *Biol Zentr* 58, 12 (1938)
94 Law, L W *Proc Soc Exptl Biol Med* 40, 442 (1939)
94a Lee, T Y *Ann Entomol Soc Am* (1948, in press)
95 Lelu P Les corrélations humorales chez les invertébrés Gauthier-Villars Paris, 1938
96 Loewe S *Naturwissenschaften* 19, 775 (1931)
97 Loewe, S , Raudenbusch, W , Voss, H E , and van Heurn, J W C *Biochem Z* 244, 347 (1932)
98 Martin, W E *Proc Indiana Acad Sci* 51, 267 (1942)
99 Mauser F *Biol generalis* 14, 179 (1938)
100 Melampy R M , and Stanley, A J *Science* 91, 457 (1940)
101 Mellanby K *Parasitology* 30, 392 (1938)
101a Mellanby, K *Parasitology* 31, 193 (1939)
102 Nabert A *Z wiss Zool* 104, 181 (1913)
103 Nyst, R H *Ann soc zool Belg* 72, 74 (1941)
103a Nyst R H *ibid* 73, 150 (1942)
104 Ochsé W *Rev suisse zool* 51, 1 (1944)
104a Paul, H *Arch Entwicklungsmech Organ* 136, 64 (1937)
104b Pautsch, F *Nature* 158, 344 (1946)
105 Pérez quoted from Wheeler (178)
106 Perez, Z *Anales Fac Ciências Porto* 25, 1 (1940)
107 Petrovskaja, O A *Biull eksp biol med* 11, 45 (1941) (Russian)

108. Pfeiffer, I W. *J. Exptl Zoöl* **82**, 439 (1939)
109 Pfeiffer, I W *Anat Record Suppl* **78**, 39 (1940)
110 Pfeiffer, I W *Trans Connect Acad Art Sci* **36**, 489 (1945)
111 Pfeiffer, I W *J Exptl Zoöl* **99**, 183 (1945)
112 Pflugfelder, O *Z wiss Zool* **149**, 477 (1937)
113 Pflugfelder, O *ibid* **150**, 451 (1938)
114 Pflugfelder, O *ibid* **151**, 149 (1938)
115 Pflugfelder, O *ibid* **152**, 159 (1939)
116 Pflugfelder, O *ibid* **152**, 384 (1939)
117 Pflugfelder, O *ibid* **153**, 108 (1940)
118 Pflugfelder, O *Biol generalis* **15**, 197 (1941)
119 Piepho, H *Biol Zentr* **58**, 356 (1938)
120 Piepho, H *ibid* **58**, 481 (1938)
121 Piepho, H *Naturwissenschaften* **27**, 675 (1939)
122 Piepho, H *Biol Zentr* **59**, 314 (1939)
123 Piepho, H *Arch Entwicklungsmech Organ* **141**, 500 (1942)
124 Piepho, H *Naturwissenschaften* **31**, 329 (1943)
125 Plagge E *Biol Zentr* **58**, 1 (1938)
126 Plagge, E *Ergeb Biol* **17**, 105 (1939)
127 Plagge, E , and Becker, E *Biol Zentr* **58**, 231 (1938)
128 Poulson, D F *Trans Connect Acad. Art Sci* **36**, 449 (1945)
129 Prell, H *Zool Jahrb allg Zool Physiol* **35**, 183 (1915)
130 Priebatsch, J *Z vergleich Physiol* **19**, 453 (1933)
131 Radtke, A *Naturwissenschaften* **30**, 451 (1942)
132 Ranzi, S *ibid* **27**, 660 (1939)
133 Reiff, M *Rev suisse zool* **52**, 155 (1945)
134 Rempel J G *J Exptl Zoöl* **84**, 261 (1940)
135 Richards, A G , Jr *J New York Entomol Soc* **45**, 149 (1937)
136 Scharrer, B *Naturwissenschaften* **25**, 131 (1937)
137 Scharrer, B *J Comp Neurol* **74**, 93 (1941)
138 Scharrer B *Physiol Revs* **21**, 383 (1941)
139 Scharrer, B *Proc Soc Exptl Biol Med* **60**, 184 (1945)
140 Scharrer, B *Endocrinology* **38**, 35 (1946)
141 Scharrer, B *ibid* **38**, 46 (1946)
142 Scharrer, B Unpublished
143 Scharrer, B , and Hadorn, E *Proc Natl Acad Sci U S* **24**, 236 (1938)
144 Scharrer, B , and Scharrer, E *Biol Bull* **87**, 242 (1944)
145 Scharrer, E , and Scharrer, B *Physiol Revs* **25**, 171 (1945)
146 Schmieder, R G *Anat Record* **84**, 514 (1942)
147 Schrader, K *Biol Zentr* **58**, 52 (1938)
148 Schwartz, V *Naturwissenschaften* **28**, 399 (1940)
149 Schwerdtfeger, H *Arch exptl Path Pharmakol* **163**, 487 (1932)
150 Steidle, H *Arch exptl Path Pharmakol* **157**, 89 (1930)
150a Steopoe, J , and Dornesco, G T *Arch zool exptl et gén* **78**, 99 (1936)
151 Tatum, E L *Proc Natl Acad Sci U S* **25**, 486 (1939)
152 Tatum, E L , and Beadle, G W *Biol Bull* **77**, 415 (1939)
153 Tatum, E L , and Beadle, G W *Science* **91**, 458 (1940)
154 Tatum, E L , and Haagen Smit, A J *J Biol Chem* **140**, 575 (1941)
155 Thomsen, E *Nature* **145**, 28 (1940)
156 Thomsen, E *Vidensk Medd Dansk Naturhist Forening* **106**, 317 (1942)

157 Thomsen, M *Kgl Danske Videnskab Selskab Biol Medd* **19**, (4) 1–38 (1943)
158 Townsend, G F, and Lucas, C C *Science* **92**, 43 (1940)
159 Vandel, A *Rev gén sci* **51**, 60 (1940/41)
160 Vogt, M *Biol Zentr* **60**, 479 (1940)
161 Vogt, M *Arch Entwicklungsmech Organ* **140** 525 (1940)
162 Vogt, M *Naturwissenschaften* **29**, 80, (1941)
162a Vogt, M *ibid* **29**, 725 (1941)
163 Vogt, M *Biol Zentr* **61**, 148 (1941)
164 Vogt M *ibid* **61**, 242 (1941)
164a Vogt, M *Naturwissenschaften* **30**, 66 (1942)
165 Vogt M *ibid* **30**, 470 (1942)
166 Vogt M *Arch Entwicklungsmech Organ* **141**, 424 (1942)
167 Vogt, M *ibid* **142**, 131 (1942)
167a Vogt M Biol Zentr **62**, 149 (1942)
168 Vogt M *Naturwissenschaften* **31**, 200 (1943)
169 Vogt, M *Biol Zentr* **63**, 56 (1943)
170 Vogt, M *ibid* **63**, 395 (1943)
171 Vogt, M *ibid* **63**, 467 (1943)
172 Vogt, M *Naturwissenschaften* **32**, 37 (1944)
172a Vogt, M *Nature* **157**, 512 (1946)
173 Von der Wense T Wirkungen und Vorkommen von Hormonen bei wirbellosen Tieren Barth Leipzig 1938
174 Weed (Pfeiffer), I G *Proc Soc Exptl Biol Med* **34**, 883 (1936)
175 Weed (Pfeiffer), I G *ibid* **34**, 885 (1936)
176 Welsh, J H *Quart Rev Biol* **13**, 123 (1938)
177 Weyer, F *Zool Anzeiger* **112**, 137 (1935)
178 Wheeler W M *J Exptl Zoöl* **8**, 377 (1910)
179 Whiting P W, and Whiting A R *J Genetics* **29**, 311 (1934)
180 Wigglesworth, V B *Quart J Microscop Sci* **77**, 191 (1934)
181 Wigglesworth V B *ibid* **79**, 91 (1936)
182 Wigglesworth, V B *Naturwissenschaften* **29**, 301 (1939)
183 Wigglesworth V B The Principles of Insect Physiology, Dutton New York and London 1939
184 Wigglesworth V B *J Exptl Biol* **17**, 201 (1940)
184a Wigglesworth V B *Nature* **159** 872 (1947)
185 Williams C M *Anat Record Suppl* **78**, 99 (1940)
186 Williams C M *Biol Bull* **82**, 347 (1942)
187 Williams C M *ibid* **90** 234 (1946)
187a Williams C M *ibid* (in press)
187b Williams C M *Anat Record* (in press)
188 Wolf O M *Anat Record* **44**, 206 (1929/30)
189 Yakhontov V V *Compt rend acad sci U R S S* **46**, 127 (1945)

Attention is called to the Conférence scientifique internationale sur l endocrinologie des arthropodes which took place June 17 24 1947 at Paris The printed reports of the topics discussed at this conference were not available to the author at the time this book went to press

Chapter V

Hormones in Crustaceans

By FRANK A BROWN, Jr

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I Introduction

A number of hormones, produced at specific points within the body and transported in the blood, have now been satisfactorily shown to be

concerned in coordination and integration in crustaceans There is, furthermore, strong suggestion that still other processes are normally influenced by hormones, though reasonable proof of these latter is still lacking It appears that the same general types of functions are controlled or influenced by hormones in the crustaceans as in the vertebrates Included in a list of such functions are color change, molting and growth, certain aspects of general metabolism, and differentiation and maintenance of sex characteristics The sequence in this list is also roughly the order of decreasing extent of our knowledge regarding the details of the total hormonal mechanism which is involved In no instance has a hormone been obtained in a pure state Experimental work in no case has proceeded beyond studies of the results of extirpation of tissues or organs containing the source of hormones, implantations of these tissues or organs, blood transfusions, or injections of either crude extracts of the glandular tissue or of partially fractionated extracts still probably containing a wide variety of substances Despite this, there has accumulated a fairly considerable body of information as to the roles that certain endocrine tissues and their hormones play in the economy of the individual In the absence of chemical isolation or purification of the active principles, however, it is frequently very difficult to delineate the exact role of each hormone by itself Hence it has not been possible, in general, to demonstrate the identities of similarly acting hormones from different species and to apply names to these principles with any real degree of assurance

Compared with the state of our knowledge of vertebrate hormonal mechanisms our knowledge of those of the crustacean is in a most elementary and fragmentary state

A number of reviews have been written on the general subject of invertebrate hormonal mechanisms (62,65,91,93,97,127,154) Other and more recent reviews have been restricted to crustaceans (27,30,81)

II Hormones and Sex Characteristics

A General

The malacostracan crustaceans, in general, are dioecious and show a distinct sexual dimorphism, with the two sexes readily distinguishable on the basis of a number of characteristics The first suggestion of a hormonal activity within crustaceans came from observations on the development and maintenance of these secondary sexual characteristics, and was first called forth as an hypothesis to explain the results of parasitic castration (50) of male decapod crustaceans [1] The earlier observations are

[1] *Cf* Section III D of the preceding chapter (p 138) for the effects of parasitic castration in insects

ably summed up in the excellent paper of Tucker (140) Parasitic castration by rhizocephalans such as Sacculina, Peltogaster, Triangulus, and Lernaeodiscus has been described frequently These organisms, after a brief existence as free-swimming larvae, become attached to the body of their host and eventually become little more than sacs containing reproductive organs and with nutritive roots growing deeply through the host, destroying tissues and organs, and robbing host nutrients Other common parasites of crustaceans involved in castration are epicarid isopods such as Gyge and Bopyrus These latter parasites enter the branchial chamber of the host as free swimming larvae, become attached, metamorphose, and feed upon the body fluid An infestation by such parasites leads to more or less suppression, degeneration, or occasionally even destruction, of the gonads, the extent of the effect varying greatly with the host species, parasite species, and the specific case

B Male Sex Characteristics

Parasitized males commonly show incomplete differentiation of such typical secondary sexual characteristics as the specialized copulatory pleopods, the narrower abdomen, and the larger male type chelipeds These portions of the body tend to assume forms resembling more closely the homologous parts in the female

This modification of the sex characteristics has been explained in various ways by different investigators Smith (132,133,134) noted that females showed a greater rate of fat production than males and that the parasitized males resembled females in this regard Smith formulated the hypothesis that the parasite in the male imposed the same metabolic demands on the host as the normally active ovary of the female Both utilized large amounts of fats He believed that sexual formative stuffs, related in some manner to the fat metabolism, were involved in influencing ovarian or testes activity and, in parallel, the secondary sexual characteristics This hypothesis has been supported by Robson (124), Tucker (140), and Hughes (71)

Another hypothesis was that set forth by Biedl (17), who suggested that the parasite was female and liberated a feminizing hormone into the host's blood Others have criticized this view on the ground that the parasite is not a female but a hermaphrodite

The first investigator to suggest action of a male hormone was Courrier (43) working on parasitized male Carcinus He could find, however, no correlation between the degree of suppression of the gonad and the extent of influence on the secondary sex characteristics Therefore, he concluded that the male hormone must be formed in some tissue other than the gonads, and that this source must be suppressed or destroyed by the

parasite Okada and Miyashita (107), working on the crab, Eriocheir, confirmed Courrier in finding no significant correlation between the degree of suppression of the gonads and of the secondary sex characteristics Lipschutz (100), on the other hand, assumed a normal liberation by the testis of a male hormone Its absence or reduction following parasitization was considered to result in a change of the host toward a neutral form This view was also upheld by van Oordt (143,144) and was given strong support by the work of Brinkman (20), who found in an extensive study of parasitization of the male crab, Munida, with three species of rhizocephalans that there was (1) a good correlation between the degree of suppression or degeneration of the testis and the extent of modification in the female direction, and (2) no similar correlation between the size of the parasite (and hence the nutritional drain on the host) and the change Brinkman believed, however, that both a male hormone and malnutrition were involved in the explanation of the feminization

Another interpretation of the results of parasitic castration in male crustaceans was that of Goldschmidt (51), who considered the parasitized form to be an intersex as a result of an influence of the parasite upon the normal expression of the sex genes A somewhat similar point of view was adopted by Callan (38), who found no perceptible influence of castration upon the secondary sex characteristics of the male shrimp, Leander The latter investigator believed that the different species of crustaceans varied widely in the stability of their sex determining mechanism, with parasitic infestation being able to tip the balance much more readily in some than in others On the basis of this hypothesis Leander appeared to show a rather stable condition Less stable conditions were found by Potts (119), who reported that parasitized male Eupagurus showed production of ova in the testis Comparable observations were made by Smith (132) for Inachis, and Tucker (140) for Upogebia Evidence for a normal tendency toward hermaphroditism in higher crustaceans has been reported by Runnstrom (125) and Turner (141)

Certain seasonal variations in secondary sexual characteristics in the male crayfish also suggest an action of hormones The copulatory appendages typically show a seasonal variation in form, assuming a sexually functional form at the summer molt and a nonfunctional form at the spring molt Scudamore (130) has pointed out that the time of metamorphosis to the functional condition is a time of high testis activity, according to Fasten (47), and that there is a minimum of testis activity at the time of the spring molt Scudamore also found that molts experimentally induced by removal of the eyestalks during the winter months invariably yielded the nonfunctional type, and correspondingly this also was a time of low testis activity

C Female Sex Characteristics

Female crustaceans in general do not appear to show an extensive modification of their secondary sexual characteristics upon parasitic castration as do males Potts (119) working on Eupagurus, and Miyashita (105) on Eriocheir have described a tendency of parasitized females to retain juvenile characteristics Potts also described a slight change of form of pleopod from the typically biramous type of the female toward the uniramous one of the male

The female does, however, commonly exhibit certain seasonal modifications of body form associated with breeding activity These changes may involve brood pouches, incubatory chambers, and related structures Le Roux (98,99) has observed that parasitic castration of Gammarus by a worm, Polymorphus, usually results in failure of development of characteristic marginal setae of the oöstegites These setae were similarly inhibited when ovarian activity was suppressed by irradiation but did eventually develop, along with a restored ovarian activity, following cessation of irradiation treatments Haemmerli Boveri (52) working on Asellus, and Mori (106) working on Daphnia reported that irradiation resulted in suppression both of ovaries and of brood pouch formation Callan (38) found with female Leander that parasitic castration and x ray castration were both associated with failure of the typical incubatory chamber to differentiate during the breeding season The typical pattern of white-reflecting chromatophores of the female was also absent (87) Callan leaned toward an explanation of his results in terms of the activity of a hormone arising in the ovary, but realized that other interpretations of the results were not ruled out

Recently McVay (101) has reported finding a seasonal fluctuation in abundance in female crayfish of a chromatophorotropic factor from the brain concentrating white pigment Males and females showed similar quantities during the nonbreeding period, while females possessed substantially less during breeding activity

D General Conclusions

We may sum up by saying that many observations suggest strongly that hormones are operative in the development and maintenance of male and female sexual characteristics Such a suggestion arises chiefly from the often demonstrated fact that suppression or degeneration of the gonads following parasitic castration or irradiation is commonly associated with more or less modification of the sex characteristics in the direction of either a neutral form or of the opposite sex The extent and character of the modifications vary with the host and the parasite species and with the individual case Crucial experiments have not yet been

performed to enable us to conclude definitely that specific sex hormones are actually operating, and, if so, in what tissues they arise Resolution of this problem must await results of conventional endocrinological experiments involving studies of the effects of tissue extirpations and replacements, and the isolation and physiological study of the active principles

III Hormones and Color Changes[1]

A General

The first decisive demonstration of hormonal activity in the crustaceans came from a study of the controlling mechanism of physiological color changes, and it is on this subject that the largest amount of research on crustacean hormones has been done Just as a similar approach to vertebrate hormonal mechanisms would have probably soon led to two very important hormone sources, the adrenals and the pituitary, so it happened that this attack on the crustacean led to two rich sources of hormones One of these, the sinus gland, usually within the eyestalks has already been shown to possess a number of important functions other than control of color change Other sources, within the central nervous organs, also appear to give some indication of possessing functions within the organism of more fundamental importance than control of color changes

B The Chromatophores and Their Normal Activity

Color changes in the crustaceans are brought about by the activities of chromatophores The earlier literature on this subject has been extensively reviewed (6,48,112) The chromatophores comprise numerous small syncytial bodies in the hypodermis, or directly beneath it, and over certain of the internal organs According to the opinion now most generally held, these bodies have diffusely branched, radiating processes of a permanent character Within a single animal the chromatophores over the body may contain pigments of one or, more commonly, several different colors Crustacean pigments include a black or sepia (particulate melanin), reds and yellows (carotinoids), blue (a carotinoid-protein complex), and reflecting white (particulate guanine) Each pigment within a chromatophore possesses the capacity of (1) moving centripetally to form a small mass near the chromatophore center (pigment concentration), or (2) dispersing centrifugally until the whole of the chromatophore, even to the tips of its branches, is filled with the pigment (pigment dispersion), or (3) maintaining any intermediate degree of dispersion or concentration The degree to which any given pigment contributes to the gross coloration of the animal is a function of the degree of dispersion

[1] Corresponding phenomena in the vertebrates will be treated in Volume 2

of that pigment within the chromatophore Only the blue pigment commonly appears outside of chromatophores where it often seems to pervade the general body tissues

The chromatophores may be classed as monochromatic, dichromatic, or polychromatic depending upon the number of types of pigment found within each When more than one pigment is present within a single chromatophore, these pigments usually remain separated from one another and usually each possesses its own chromatophore branches into which it disperses

The chromatophore system of many crustaceans possessing transparent or translucent cuticles constitutes a very effective mechanism for enabling the individual to mimic rather closely the shade, and often even the tint, of the background upon which it comes to lie The common shrimp, Palaemonetes, possesses within its chromatophore system red, yellow, blue, and white pigments By the appropriate differential dispersion or concentration of these four pigments in sympathetic response to backgrounds, the animal is able to assume any spectral color and may even closely approximate black on the one hand, or almost complete transparency on the other The shrimp, Hippolyte, appears to possess, in addition to these abilities, power to assume adaptive color patterns as well (49,104) The sand shrimp, Crago, which contains black brown, red, yellow, and white pigments, lacks the ability to assume bluish or greenish tints though displaying otherwise considerable powers of modifying its shade, tint, and general pattern of coloration to conform to its background (89) Such remarkable powers of chromatic adjustment are obviously possible only in terms of relatively independent control, on the part of the animal, of each of its various pigments

The chromatophore system also typically responds to changes in light intensity, usually assuming one state characteristic for the species during darkness at night, and another in light during the daylight hours In some species this appears to be exclusively determined by the light intensity change while in others the response is strongly conditioned or even determined by an inherent diurnal rhythm within the animal In the latter instances the typical diurnal changes may proceed even under conditions of a constant state of illumination and background A species showing the former type of response seems to be Palaemonetes, where the animal on a black background pales in darkness and darkens in light without relation to the diurnal cycle The fiddler crab, Uca, on the other hand, normally pales at night and darkens by day despite the maintainance of constant illumination and background The latter species shows relatively little adaptive response to color of background in light

C Hormonal Control of Chromatophores

1 *General Historical Background*

It was assumed for many years that the chromatophores of crustaceans were directly innervated organs, though it gradually came to be realized that no one had demonstrated histologically any nerve endings at these organs, nor could workers show that the types of nerve transection which they performed interfered significantly with color changes Koller (88,89) working on the shrimp *Crago vulgaris*, was the first investigator to provide positive evidence that controlling agents for crustacean color changes are carried in the blood Koller s experiments involved transfusion of blood from one animal to another He noted that when blood from a black animal was transfused to a white one, the latter darkened even though kept upon a white background Blood from a white donor had no such effect, though neither did it lighten black recipients maintained upon a black background Blood from a yellow-adapted animal induced distinct yellowing of a white animal These blood transfusions brought about the color changes at approximately the same rate as normally followed the corresponding background changes

Perkins (116) found no evidence whatsoever that the chromatophores of the shrimp, *Palaemonetes vulgaris*, were under the control of nerves He could discover no direct nerve innervation, nor would extensive nerve transection experiments interfere with the color changes in this form When, on the other hand, blood flow in the dorsal abdominal artery supplying the posterior portion of the body was stopped, color change posterior to the point of stoppage immediately ceased Later when blood flow resumed, the posterior portion of the body quickly assumed the color of the remainder of the body Perkins interpreted these results as due to blood borne factors inducing pigment concentration and dispersion In attempting to determine the origins of these hormones, he extracted separately, in sea water numerous organs and tissues of the body and observed the action of injections of these extracts into black- and white adapted individuals Of the numerous extracts tested, only one—extracts of the eyestalks—resulted in lightening of dark specimens, and none produced darkening of light individuals Perkins also found that extracts of eyestalks from white-adapted donors were much more effective than those from black-adapted ones Animals from which the eyestalks were removed darkened and remained so permanently On the basis of these experiments Perkins concluded that the eyestalks contained the source of a hormonal substance which lightened the body through concentration of the red and yellow pigments

These results were completely confirmed for Crago, Leander, and

Processa by Koller (90), who also demonstrated that the eyestalk hormone was not species or even genus specific Koller sought further for the source of the blood borne principle which resulted in the darkening of white-adapted Crago which received a blood transfusion from a black one He found that injection of extracts of the rostral region of black adapted Crago or Leander caused darkening of white-adapted Crago White adapted animals darkened after feeding on the rostral region Cautery destruction of this region deprived animals permanently of the ability to darken again Koller concluded that an endocrine gland was located in the rostral region and that it produced a principle influencing the dark pigments of the body antagonistically to one from the eyestalk

Following these initial demonstrations of hormonal control of crustacean color changes, many crustaceans were examined to determine how generally the hormonal activity was present within the group The eyestalks, or occasionally the heads instead, of some seventy to eighty species of crustaceans were shown to yield sea water extracts with strong chromatophorotropic activity upon the chromatophores of injected animals This activity was generally similar to that which had been found in the eyestalks of Palaemonetes and Crago The chromatophorotropic material appears so commonly present among the higher crustaceans that the three isopods, Oniscus Porcellio and Mesidothea reported not to have it (137,138) should certainly be thoroughly re-examined

Attempts to repeat Koller s observations on the rostral region gland have met with almost uniformly negative results Beauvallet and Veil (12) working on Leander reported confirmation of Koller's rostral gland, but Panouse (109) and Carstam (41) also using Leander failed to confirm it Attempts to discover a rostral gland for Palaemonetes by Perkins and Snook (117) and Brown (22), for Cambarus by Hanstrom (60), and even to confirm the presence of one in Crago itself by Kropp and Perkins (95), Kleinholz (80), Panouse (109), and Carstam (41) all led to negative results It appears therefore that no endocrine gland with the function ascribed to it by Koller lies in the rostral region There is now reason to suspect that a hormone originating in anterior central nervous organs was responsible for the positive results which were obtained occasionally This latter possibility will be considered later in Section 3 page 175

Numerous observations have been made of the effects of extirpation of the eyestalks upon the state of the chromatophores in a number of species of crustaceans It is unfortunate for the interpretation of the results that the operation removes not only the gland in question (see Section 2 below), but also important central nervous organs and the principal photoreceptors known to be essential to the normal reflex color adaptations of the animals The observations at hand suggest that

crustaceans fall into three groups (Fig 1) with respect to the character of their response to eyestalk removal One group, exemplified by the shrimp, Palaemonetes, includes the majority of Isopoda, Mysidacea, Natantia, and Astacura which have been investigated The dominant dark pigments of these animals disperse widely, yielding a permanently darkened condition of the body Injection of eyestalk extract into these eyestalkless animals induces rapid lightening

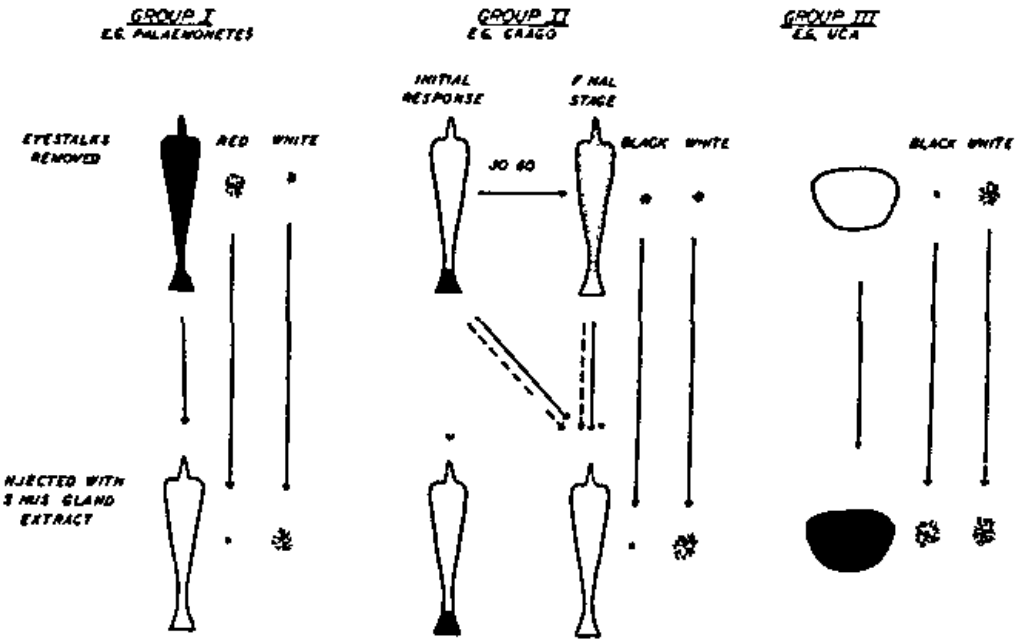


FIG 1.—Diagrammatic representation of the results of removal from crustaceans of the eyestalks with their included sinus glands upon the coloration and dominant chromatophore types (top row) All crustaceans so far investigated fall into one or another of the three groups The bottom row shows the influence of injection of eyestalk or of sinus gland extracts from other animals of the same group into the eyestalkless specimens Dotted arrows indicate an alcohol soluble fraction only, dashed arrows indicate only an alcohol insoluble fraction Reciprocal injection experiments among the three groups show that crustaceans of group III possess no telson- and uropod lightening activity for Crago of group II, but otherwise there are no qualitative differences

A second type of response is found in Crago *Eyestalkless Crago* show an intermediate and mottled coloration (24) Some of the dark chromatophores have their pigment broadly dispersed, others are in an intermediate condition, while still others have theirs fully concentrated These animals respond to eyestalk extract injection by uniform blanching

A third type of response, exemplified by the crab, Uca, is exhibited by all the Brachyura (true crabs) which have been investigated Eyestalk removal in these yields a permanently pale condition of the body due to maximum concentration of the dominant dark pigment (1,39)

Injection of eyestalk extract results in rapid darkening of the body, the reverse response to that seen in the first two types

The state of the pigments following eyestalk removal seems to differ in various crustaceans, with each species possessing its own characteristics In view of this situation and the fact that reciprocal injection experiments seemed to suggest that regardless of the species contributing the eyestalks, their extracts would call forth the same reaction as an extract of stalks from the same species, Abramowitz (4) proposed the hypothesis that all the crustacean pigmentary behavior could be explained through the action of one hormone, eyestalk hormone—ESH of Abramowitz and Abramowitz (8) The differences in response among species were believed explainable in terms of differences in the thresholds and in the characters of response of the various chromatophores to the single hormone This concept became known as the "unitary hormone hypothesis" and has been supported by a number of investigators

In contrast with the unitary hormone hypothesis was the 'multiple" one According to this concept all the observed pigmentary responses could not be explained in terms of a single chromatophorotropic principle This view was implied in the work of Perkins (116) by his factors for concentration and dispersion, and definitely supported by Koller (89,90,92) with his work on the eyestalk hormone the rostral-region hormones, and his yellow factor from elsewhere in the body Also the work of Koller (89) on Crago, Brown (22) on Palaemonetes, Abramowitz (1) and Hitchcock (69) on the crab, Portunus, showed that in adaptation of these animals to colored backgrounds, various combinations of pigments displayed ability to distribute themselves within the chromatophores more or less independently of one another Such relatively independent activity of the pigments had been known for many years to be true for the shrimp, Hippolyte (Keeble and Gamble, 74, and Minkiewicz, 104) Brown (22) proved by nerve transection experiments that the independent activity of the four pigments of Palaemonetes was wholly the result of hormonal action and suggested that at least four chromatophorotropic hormones were present to account for the observed phenomena Parker (114) pointed out that three principles would account for the behavior in this species Smith (135) by very ingenious experimentation has presented evidence for separate body lightening (W factor) and body-darkening (B factor) principles in an isopod Carstam (41), working on Leander, demonstrated separate controlling factors for the red and yellow pigments in this shrimp

In addition to the preceding work, several experiments indicated that chromatophorotropically active substances could also be extracted from central nervous organs of crustaceans Brown (21) discovered that

extracts of these organs of Palaemonetes would induce paling of dark, eyestalkless animals though no other organ of the body except the eyestalks would do likewise This observation was confirmed for two species of Penaeus by Hosoi (70) and Hanström (60) Knowles (86) found that extracts of central nervous organs concentrated white pigment in Leander Brown (22) noted that the dark and white pigments of eyestalkless Palaemonetes could be made to concentrate within the chromatophores in response to electrical or heat stimulation of the cut ends of the optic nerves This last observation found a reasonable interpretation in the activity of hormonal material originating in the central nervous organs

2 *The Sinus Gland*

a Structure and Innervation Hanström in 1933 (54) described for the first time in the crustacean eyestalk a gland which he first called the blood gland but later (60) named the sinus gland Since that time the gland has been found in all the higher crustaceans in which it has been

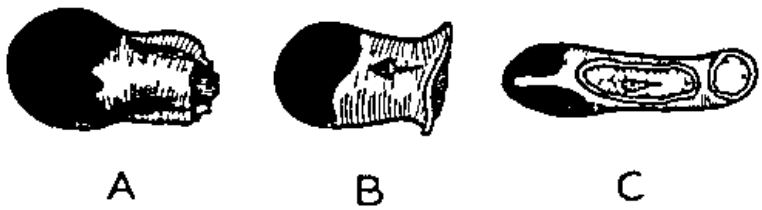


FIG 2 —The sinus glands in the eyestalks of A, Palaemonetes B, Crago and C, Uca, as seen from the dorsal view In species such as Crago and Palaemonetes possessing transparent cuticles the gland is clearly visible in the intact living specimen in which it appears as a bluish white opaque organ against the more transparent grayish white underlying nervous tissue (Modified from Brown, 26)

sought (26,31,41 60,131,138) In the vast majority of the stalk-eyed crustaceans it lies in the eyestalk (Fig 2) In some stalk-eyed species (*e g* , Upogebia and Emerita) and in species without eyestalks the gland lies close to the supraesophageal ganglion in the head In the decapod crustaceans upon which most experimental work has been done the gland occupies a dorsal or dorsolateral position in the eyestalk, most commonly lying opposite a point between the medulla externa and interna Less commonly it lies opposite the medulla interna, while in a few species it has an attenuated form and occupies a position opposite the medulla interna and medulla terminalis In shrimp with highly transparent cuticles, such as Palaemonetes and Crago, the gland is clearly visible in the intact living animal held in bright incident illumination The gland possesses a more bluish-white coloration than the remainder of the stalk tissue, probably due to the large amount of intracellular inclusions of the gland

cells In species with thick opaque cuticles the gland may be seen in fresh tissue by dissecting away the dorsal exoskeleton and hypodermis of the stalk The gland occupies less than $\frac{1}{100}$ the volume of the eyestalk in the crayfish, Cambarus (31)

Hanström (60) believes that the gland originates phylogenetically as a thickening of the neurilemma over the nervous elements of the eyestalks, with its simplest and most primitive form found in certain mysids, euphausids, isopods, and amphipods In the Natantia with few exceptions, the gland occupies a portion of the neurilemma at a point where a blood sinus within the central nervous system opens into the large superficial sinus of the stalk and thus the gland possesses a beaker-shaped form In the Astacura the inner blood sinus has become complexly branched, and, with this, the sinus gland which occupies its walls In most of the Brachyura examined, the gland has the form of a hollow sphere Here the primitive gland is believed to have separated from the neurilemma, become invaginated, and liberate its products into the lumen which is connected with both the inner and the outer blood sinuses In those decapods in which the gland is in the head instead of the eyestalks, as in certain anomurans, the gland appears secondarily simplified to form a simple plate of glandular tissue in contact with only an outer blood sinus into which the contents appear to be discharged directly

The cells comprising the sinus glands appear richly charged with acidophilic inclusions, staining with eosin acid fuchsin, and light-green (Hanström, 60) Also described are basophilic inclusions with the relative abundance of the two types of granules varying with the different stages in the molting cycle in the crayfish, Cambarus (120) Hanstrom (60) also described for the cells fine secretory canals for the conductance of gland products to the sinus

The gland is richly innervated It is supplied on its inner surface by a large nerve arising in the medulla terminalis (60), and at least in Cambarus some fibers of this nerve appear to arise in the supraesophageal ganglion (153) In this latter species Welsh (153) has also described a branch of the oculomotor nerve passing to the region of the gland Thus, the gland appears to have a triple innervation

b Chromatophorotropic Activity Shortly following upon the demonstration that the eyestalks of crustaceans produced hormonal material active upon chromatophores came attempts to localize the source within the stalk Koller (92) divided the stalks of Crago transversely into two portions, the sensory portion and the remainder Since the sensory portion showed slight activity, even though by far the greater part of the activity lay in the remainder of the stalk, Koller concluded that the blood gland at the base of the retina was the source Destruction of this sup-

posed source by cautery produced lasting body darkening, therefore adding apparent confirmation to his conclusion

Hanstrom (60) believed that Koller's blood gland could not be the actual source of the active material since it was not present in the chromatophorotropically active eyestalks of some species such as Palaemonetes Furthermore, the gland was not innervated as would perhaps be expected Nor did Hanstrom believe that the source of the hormone in question was the X-organ of the crustacean eyestalk (18,41,53,54,55,56,57,58,60, 66,138) even though this organ appeared to possess the histological characteristics of an endocrine gland The X-organ, also, was not found in a few species bearing active eyestalks (*e g* , Astacus, Uca) On the other hand, Hanstrom's sinus gland was found in all the numerous malacostracan crustaceans in which it was sought; its cells showed every indication of active secretory activity, and it was well innervated as it seemed reasonable to expect in view of the reflex nature of crustacean color changes

Hanstrom (59,60) carried out an extensive survey of a wide variety of species of crustaceans in which the eyestalks, or heads of those species in which the eystalks were inactive, were sectioned in various ways and the portions extracted and injected into species with active, readily observable chromatophores His assay animals consisted in different experiments of Palaemonetes, Uca, or Penaeus In these experiments Hanstrom utilized in a very ingenious manner the species differences in the position of the sinus gland with respect to other organs in the eyestalk or head He showed quite conclusively that every active portion always contained the sinus gland in whole or in part and that no inactive portion ever did Furthermore, he managed by judicious selection of species to get one by one every conspicuous organ of the typical crustacean eyestalk into a portion without the sinus gland and found extracts of each one in turn to be inactive The sinus gland therefore appeared to be the exclusive source of hormonal material blanching the bodies of dark shrimp on the one hand, and darkening the bodies of pale fiddler crabs on the other

These conclusions were fully confirmed by Brown (26), who removed the sinus glands by themselves from a number of crustaceans Callinectes (blue crab), Carcinides (green crab), Crago, Libinia (spider crab), Pagurus (hermit crab), Palaemonetes, and Uca The activities of extracts of the glands by themselves were compared with extracts of the remainder of the eyestalks in their action in concentrating the red pigment of Palaemonetes on the one hand and dispersing the black pigment of Uca on the other It was found that approximately 80% of the activity of the whole eyestalks was present in the sinus glands which occupied less than 1% of the total volume of the stalks In dissecting

out the sinus glands from the stalks a bluish-white cloud of colloidal material could usually be seen to pass out of the gland into the surrounding tissues Such an escape of substance could reasonably be expected to account for the remaining 20% of the activity seen in the residual stalk tissue It was found, furthermore, that the activity of the glands by themselves was the same as that of the remaining stalk tissue in relative effectiveness upon the two types of chromatophores, further suggesting the gland as being the sole source in the stalk of hormonal material influencing these two chromatophore types Still further confirmation was also found in the action of implants of sinus glands in the ventral abdominal sinus of Palaemonetes A single implant maintained the red pigment in eyestalkless specimens more or less concentrated for as long as five days, *i e*, many times as long as that ever found following injection of highly concentrated extracts of eyestalks

The only attempt that has been made to remove the sinus glands alone from the eyestalks for the study of chromatophoric responses (33) involved the bilateral removal of the glands by microaspiration from several specimens of Palaemonetes, Such sinus glandless animals became dark and showed no ability to concentrate their dark pigments in response to white backgrounds Proof that the glands were completely removed was afforded by injecting into test animals extracts of the stalks of sacrificed animals

The sinus gland has also been shown to affect other types of pigments than the two mentioned above Each of the eight physiologically different pigments of Crago shows its own response to injection of sinus gland extract (32,37) The pigments are induced to concentrate, or disperse, to different relative extents The white pigment of Cambarus has been shown to disperse, and the red to concentrate, under the influence of sinus gland extracts (34)

From the preceding account it is seen that much evidence exists that the sinus glands are chromatophorotropically active, and there is no evidence that any other eyestalk organ is active in this regard

c The Number of Principles and Their Activities The eyestalks, or the sinus glands by themselves, yield hormonal material which upon injection produce within the shrimp at least grossly the same response (body lightening) as that normally called forth by a white background in light, and within true crabs the typical dark coloration seen in the day time phase of their diurnal cycle (4) The removal of the eyestalks from the shrimp brings about a state of the coloration which tends in the general direction of that seen in normal response to an illuminated black background, and in the crab to a condition simulating the nighttime phase of its diurnal cycle However, the coloration of eyestalkless

shrimp is distinguishable from black-adapted ones Palaemonetes always remains more reddish-brown, and it will be recalled that Crago simply reaches finally an intermediate mottled coloration Supporters of the "unitary hormone hypothesis" discussed earlier assumed that a single chromatophorotropic hormone (ESH) was produced by the sinus gland However, in view of the overwhelming weight of the evidence indicating that two to several chromatophorotropic hormones must be present within the animals, there seemed to be a reasonable possibility that the sinus gland itself was responsible for more than one of them, especially since all of the numerous pigmentary types investigated among crustaceans were shown to be affected by extracts of this gland

Brown and Scudamore (36) sought to determine whether or not a single hormone from the sinus gland is responsible for all of the observed reactions of the chromatophores to extracts of the gland They made a comparative survey of the effects of eyestalk and sinus gland extracts from Crago Carcinides, Libinia, Uca, Pagurus, Callinectes, and Palaemonetes simultaneously upon Uca black, and Palaemonetes red, pigments The ratio, (effect upon Uca-black)/(effect upon Palaemonetes-red), differed in a repeatable manner with the species source of the gland The order of decreasing size of the ratio was the order of species listed above This order bore no relationship either to the relative weights of the animals or to the apparent relative concentrations of ESH as determined by Abramowitz (3) upon Uca, which were respectively in grams, 1, 60 50, 2, 11, 100, 1, and in Uca units 0 25 1 25, 4 0, 1 0, 1 25,—, 0 36 A hypothesis that the differences observed for the ratios were the result of differing concentrations of two factors, a Uca-black-dispersing hormone and a Palaemonetes-red concentrating hormone, was fully borne out by the partial separation of the extract into two fractions, one relatively soluble in 100% ethyl alcohol and the other relatively insoluble in this solvent The alcohol insoluble fraction showed a significantly higher Uca/Palaemonetes ratio and the alcohol-soluble fraction showed a distinctly lower one than sea water extracts of whole glands Furthermore a ratio closely approximating that shown by whole-gland extract was re obtained by mixing the two fractions These results seem most reasonably explained in terms of possession by the glands of two different hormones with the glands of the seven species examined varying in the relative amounts of the two present

Evidence from studies of the comparative influence of sinus gland extracts upon a third chromatophore type, the melanophores of the telson and uropods of Crago by Brown and Ederstrom (32) point to the presence of still a third principle Sinus gland extracts from Crago and Palaemonetes, but not of Uca and Carcinides will produce a very powerful and

rapid concentration of this pigment The effectiveness of the extracts in bringing about this action appears to bear no relationship to the ability of the extracts to influence either Palaemonetes red pigment, on the one hand, or Uca black, on the other Therefore it appears that sinus glands of Crago and Palaemonetes contain a hormonal substance not present in significant amounts in the glands of the crabs Brown and Wulff (37) showed that this hormone remains in the alcohol insoluble fraction of those sinus glands which possess it (Fig 1, page 168)

For the purposes of convenience of reference and also in order to focus attention upon three differently acting chromatophorotrophic principles of the sinus glands of crustaceans it is proposed at this time that they be named (1) Palaemonetes lightening hormone or PLH, (2) Uca-darkening hormone, or UDH, and (3) Crago-telson lightening hormone or CTLH These terms are not applied with any intended implication that these three together necessarily constitute the total of the sinus gland hormones which influence chromatophores nor that it is expected that it will be found eventually that any single hormone influences exclusively a single chromatophore type On the contrary, it seems reasonable to expect that other principles will be found in the glands, with single ones influencing more than one type of pigment cell

3 *Chromatophorotropic Hormones from Central Nervous system*

Crustaceans from which the eyestalks with their included sinus glands have been removed normally reach a condition of the chromatophores characteristic for each species and which they maintain within rather narrow limits for an indefinite period, if the animals are not disturbed These eyestalkless animals are, nevertheless, capable of showing changes in the state of their chromatophores upon appropriate stimulation of the stubs of the optic nerves These changes in those cases which have been analyzed for the means of chromatophore influence indicate that the changes are due to activity of blood borne agents

Uca, after eyestalk removal, continue to show a diurnally rhythmic activity of the dark pigment though in greatly reduced degree (5 36) Eyestalkless Hippolyte continue to respond to darkness and light by concentration and dispersion respectively of their dark pigment (49,85) Undisturbed eyestalkless Crago are occasionally seen to exhibit a transitory blackening of their telson and uropods (apparently associated with molting activity) or even of their whole body Observations such as these suggest strongly that there is a normal source of chromatophorotropic hormonal material in a tissue outside of the eyestalks, and that this source could produce coloration changes either in the same direction as those induced by sinus glands (Uca, Palaemonetes) or oppositely (Crago)

In this connection it has been found recently (unpublished) that extracts of the central nervous system of Uca exhibit great effectiveness in darkening eyestalkless animals of this species The influence of injections of extracts of central nervous systems upon the color changes of eyestalkless crustaceans is diagrammatically represented in Fig 3

The observations of Koller (90,92) and Brown (24) on Crago supported very strongly the hypothesis that a hormone antagonistic to an eyestalk-originating one lay in a region of the body other than the eye-

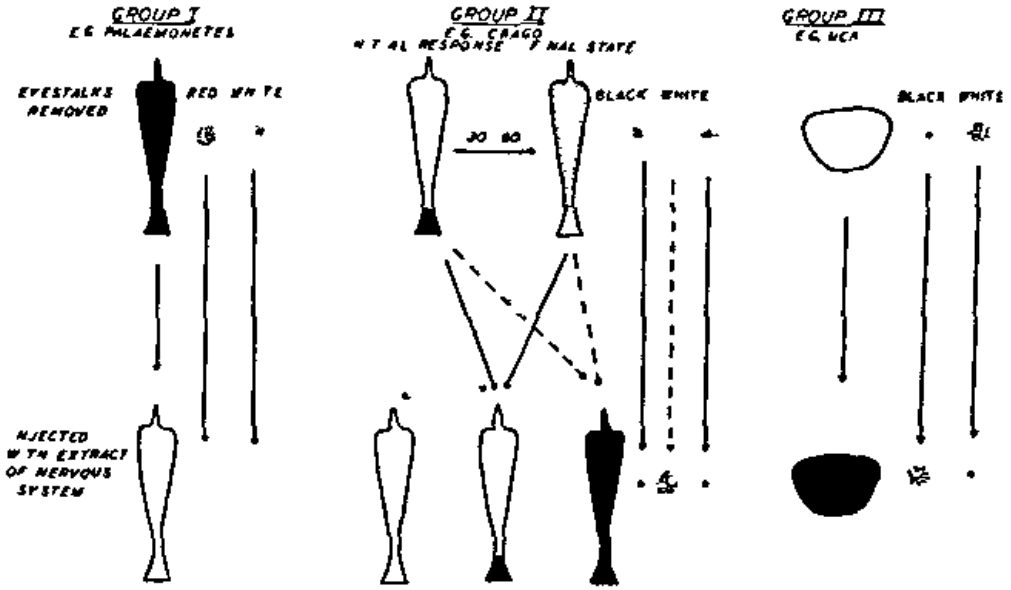


FIG 3 —Diagrammatic representation of the influence of extracts of the central nervous organs of crustaceans of each of the three differently responding groups upon the coloration and major chromatophore types of eyestalkless specimens (top row) of the same group Dotted arrows indicate action of an alcohol soluble fraction, dashed arrows indicate action of the alcohol insoluble fraction Reciprocal injection experiments among the three groups show group III to lack the body- and the telson and uropod-darkening activity for specimens of group II, but otherwise no qualitative differences seem to exist

stalks in this species It has been pointed out that Koller believed the source lay in the dorsal rostral region, in the blood cell gland located there (44,94) Stimulation of the stubs of the optic nerves in eyestalkless individuals always resulted in the blackening of the telson and uropods The rest of the body responded more variably, sometimes lightening, at other times darkening, or showed intermediate response involving an initial lightening followed by darkening (26) These responses continued after nerve transection, indicating their dependence upon blood borne agents Brown and Ederstrom (32) surveyed the tissues of Crago, injecting extracts of each into eyestalkless specimens of the same species

The midregion of the circumesophageal connectives, including the connective ganglia, was found to be most effective in blackening the telson and uropods in these animals with positive responses resulting in more than 90% of the experiments Activity of the nervous system dropped off sharply along the connectives anteriorly and posteriorly from this region, dropping off much more abruptly in black adapted than in white-adapted specimens No other tissue of the body gave a similar darkening response These workers postulated that a telson- and uropod-darkening principle was produced in the connectives ganglia or in the connectives just posterior to it A normal function of such a hormone is indicated by the fact that it is rather common to collect in the field specimens of Crago with a coloration (black "tail" and light trunk) indistinguishable from that of eyestalkless specimens injected with connective extract

These observations were extended by Brown and Wulff (37), who found that extracts of the connectives affected each of the eight differently responding chromatophore types within the species The action appeared to be supplementary to the action of the sinus glands with regard to some pigments (black brown, and red) of the body and to antagonize it with respect to others (black and red pigments of the telson and uropods and all the white pigment) The darkening action on the telson and uropods was found to reside only in an ethyl alcohol insoluble fraction of the connectives, while the rest of the activity was readily alcohol soluble Therefore two active principles appeared present in the connectives These were, in general terms (1) a telson- and uropod-darkening principle, and (2) a body lightening principle In the experiments it was not possible to obtain a telson and uropod-darkening fraction without body-lightening activity present The two general types of activity were also spatially separated within the central nervous organs Only the connectives possessed the former, but all the major parts of the system contained the latter It was suggested that if the telson- and *uropod-darkening principle* of the connectives possessed a general body-darkening action when present without the body lightening principle, an explanation would be at hand for the earlier observations of Koller and of Brown Supporting, but not proving, such an hypothesis were the observations that mild stimulation of the eyestubs of eyestalkless Crago resulted in blackening of both body and "tail," whereas stronger stimulation called forth blackening of the "tail" and simultaneous lightening of the body In terms of this concept, weak stimulation could be considered to result in a liberation of only one of the two principles, while strong stimulation would cause indiscriminate liberation of both

Further experiments aimed at localization of the source of the telson and uropod-darkening hormone of Crago were carried out by Brown (26),

who found the activity to reside almost exclusively in the tritocerebral commissure (Fig 4) lying posterior to the esophagus, and passing between the two circumesophageal connectives, together with the immediately adjacent medial aspect of the connective lying between the origin of the commissure and the connective ganglion The tritocerebral commissure by itself showed by far the greater part of the total activity indicating that in it, or on it, was the actual cellular source of the hormone Therefore practically all the activity in this regard has been localized to a relatively minute portion of the nervous system It is very suggestive that this portion of the nervous system is closely associated with the stomatogastric, or sympathetic, system in these forms, as is the case with the corpora cardiaca of cockroaches which Brown and Meglitsch (34) have shown to possess powerful chromatophorotropic activity upon crustacean chromatophores Extracts of tritocerebral commissures and the adjacent region of the connectives of Crago invariably darken the telson and uropods, but show varying degrees of body lightening followed by body darkening which may readily be interpreted in terms of the hypothesis of differing relative amounts of two hormones, (1) a body- but not a "tail" lightening one and (2) a body- and "tail"-darkening one This hypothesis was recently verified thoroughly by Brown and Klotz (33a), who were able to separate quite completely the activity in extracts of the tritocerebral commissure into two fractions, through utilization of their solubility differences in alcohol and water The alcohol soluble fraction, as earlier predicted blanched the bodies of eye-stalkless Crago, while the alcohol insoluble fraction blackened both body and "tail" (Fig 5)

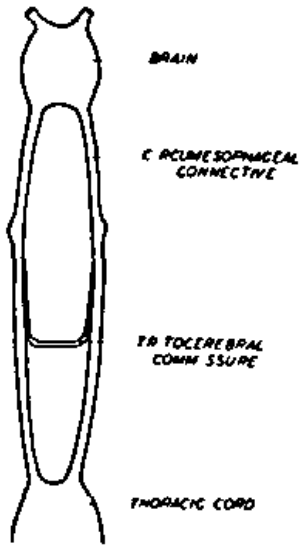


FIG 4 —A diagram showing the relationship of the tritocerebral commissure to the other parts of the anterior central nervous system of Crago

A survey of other crustaceans for the presence of the Crago-"tail"-darkening principle by Brown and Saigh (35) found no other crustacean with the commissures alone active Other crustacean groups showed (1) other regions within the nervous system active with maximum activity in the posterior thoracic cord (the anomurans, Pagurus, Emerita, and Upogebia), or (2) nearly uniform activity for all the central nervous organs (the astacurans Homarus and Cambarus and the natantian Palaemonetes), or (3) no activity in any part of the system (the brachyurans Carcinides Libinia, Uca, etc) All these other crustacean nervous systems examined, with the exception of those of the astacurans,

also showed strong Crago-body-lightening activity, this lightening activity showing in general complemental distribution to the darkening activity Homarus and Cambarus showed relatively slight lightening-activity, with certain parts of the central nervous system strongly blackening both the "tail" and body proper in eyestalkless Crago All these observations lend *further support to the hypothesis previously set forth* that the Crago-"tail" darkening principle, in the absence of an antagonistic body-lightening one is an effective body darkener

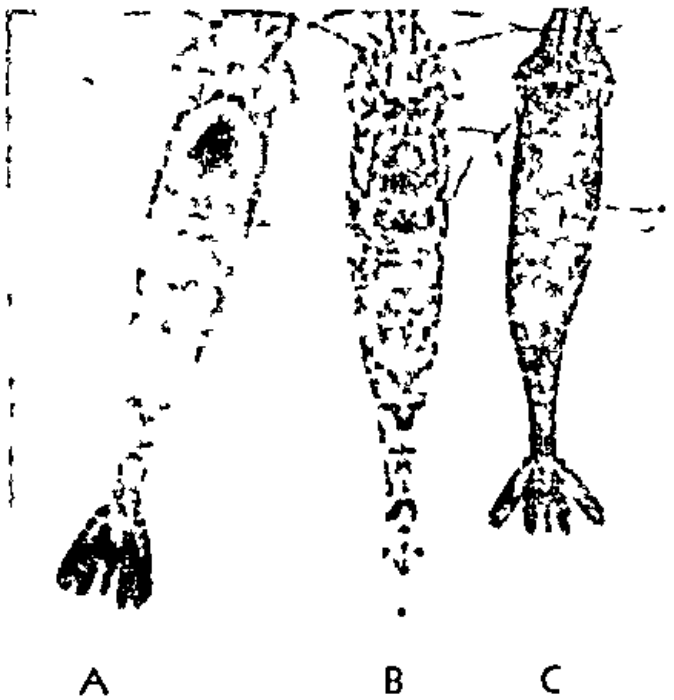


FIG 5 —A photograph showing the influence of chromatophorotropins originating in the tritocerebral commissure of the central nervous system of Crago upon eyestalkless Crago The three animals were initially matched in coloration Fifteen minutes before the photograph was taken B was injected with sea water and served as a control A and C each received injections of the equivalent of one quarter tritocerebral commissure either as a sea water extract (A) or as an alcohol insoluble fraction (C)

Evidence for the presence of two chromatophorotropic hormones within the central nervous system of Cambarus has also been obtained by McVay (101), who studied the relative effects of extracts of nervous organs upon isolated red and white chromatophores of Cambarus It is quite possible that this investigator was dealing with the same two principles previously studied

These experiments give strong evidence favoring the existence of two hormones originating from certain loci within the central nervous system It is proposed that these be called, on the basis of the responses through

which they have been differentiated (1) Crago-darkening hormone or CDH, and (2) Crago-body-lightening hormone or CBLH

4 *Properties of the Chromatophorotropic Hormones*

We still know very little of the chemical nature of the color change hormones of the crustaceans It is very evident that all the hormones are readily soluble in water and are all insoluble in such fat solvents as ether, benzene, and chloroform (2,3,5,7,40,101) Practically every investigator in the field, beginning with Koller (92), has dealt with boiled extracts, indicating that all are stable in neutral solutions during short periods of boiling In fact there are some reports of potentiation of eye stalk extract upon boiling This has been found for Palaemonetes (116) and for Cambarus (60) although denied for Callinectes and Carcinides (36,101) Chromatophorotropic activity has been found to persist in dried eyestalks for long periods, even up to several months by Perkins and Snook (117) and Hanstrom (60)

The solubilities of the color change hormones in alcohol show greater differences Some are more soluble in ethyl alcohol than others Carlson (40) and Abramowitz (3,5) found some (up to 60%) of the activity of eyestalks in darkening eyestalkless Uca to be soluble, although Brown and Scudamore (36) found this fraction (UDH) much less soluble in this solvent than a fraction (PLH) with greater influence upon red pigment of Palaemonetes Similarly, a third hormone of the sinus gland (CTLH) is relatively insoluble in alcohol The hormones of the central nervous organs similarly show differences in their solubility in ethyl alcohol, one (CDH) is relatively insoluble in this, while the other (CBLH) is quite soluble

Carlson (40) originally showed that the Uca-darkening activity of sinus glands was resistant to brief boiling in dilute HCl and NaOH but Abramowitz (3) found that longer boiling in NaOH, but not HCl, resulted in its total inactivation Brown and Suter (unpublished) dealing with a factor influencing Cambarus red pigment found that in boiling in 0 1 *N* NaOH there was potentiation during the first 45 minutes, then rapid destruction which was complete in one and a half hours

The most successful attempt at purifying one of the hormones was that of Abramowitz (7) using black pigment of Uca for assay With adsorption techniques he was able to increase the concentration of the hormone nearly two hundred times The purified substance showed reactions characteristic of amino bases

Carlson (40) found that active material from eyestalk extract would readily diffuse through cellophane, thus indicating a relatively low molecular weight

5 *Identities and Phylogenetic Distribution of the Hormones*

Of the three active principles apparently present in crustacean sinus glands, two, namely, UDH and PLH appear to be present in all of the species examined CTLH, on the other hand is abundantly present in the sinus glands of the Natantia examined and absent, or nearly so, from all brachyurans

One of the two principles occurring in the central nervous systems (CDH) is found in one or another part of all nervous systems except those of the brachyurans, while the other one (CBLH) seems to be present in all, though relatively least abundant in the Astacura

There is as yet no clear indication that any of the color change hormones are identical with those found in insect heads or corpora cardiaca (34,62,64), in Limulus central nervous system or in vertebrates although there is a certain degree of similarity in some instances Abramowitz (5) examined the action of eyestalk extract upon vertebrate chromatophores and the action of intermedin on crustacean ones He found similar but not identical action Intermedin and the eyestalk hormone influencing Uca pigment (UDH) were also shown to have many physicochemical properties in common It was not possible, however, to balance comparable doses of eyestalk extract and intermedin Furthermore, intermedin dispersed Crago black pigment (19) while eyestalk extract concentrated it

6 *Control of Secretion of the Hormones*

The secretion of hormones by both the sinus glands and the sources within the central nervous organs appears controlled jointly by two factors (1) an inherent diurnally rhythmic mechanism and (2) the reflex responses of the animals to stimulation of the compound eyes The relative importance of the two appears to vary with the species and the chromatophore type At one extreme lies Uca in which stimulation of the compound eyes by changes in light intensity or background induces relatively minor changes in the chromatophore state, while a striking, diurnally rhythmical change continues regardless of eye stimulation At the other extreme lie such crustaceans as Palaemonetes in which the state of the chromatophores is almost entirely dependent upon the light intensity and the background, with the responses mediated through the eyes

We know almost nothing of the mechanism of the 24 hour rhythm and very little more about the relationship between the compound eyes and the state of coloration The darkening and lightening responses are dependent upon the relative degrees of stimulation of dorsal and ventral

portions of the retina (4,60,63,75,135), however, it is still too soon to do more than just speculate upon just what hormones, and in what proportions, are responsible for any given state of the chromatophore system

D General Summary

The chromatophore system of crustaceans is controlled almost exclusively by hormonal substances arising within the sinus glands of the eyestalks or head, and within central nervous organs The active locus or loci within the central nervous organs varies with the species but is relatively constant within each of the major groups of decapods The sinus glands appear to possess three principles which have been named on the basis of the principal activity by which each was differentiated from the others, as (1) PLH (Palaemonetes lightening hormone), (2) UDH (Uca-darkening hormone), and (3) CTLH (Crago-"tail"-lightening hormone) The first two are found within all sinus glands tested while the third is absent, or practically so, from all the true crabs (Brachyura) The central nervous organs contain at least two active principles (1) CDH (Crago-darkening hormone), produced in the tritocerebral commissures of Crago and in other portions of the nervous system of other crustaceans (except the true crabs, from which it is entirely absent), and (2) CBLH (Crago-body lightening hormone), of general distribution through all the decapods examined, being least abundant in the astacurans, lobster, and crayfish

These hormones have not yet been identified with any noncrustacean hormones, though one, UDH, resembles intermedin in many respects The hormones are all water soluble, some are relatively soluble in ethyl alcohol, and none are soluble in the common fat solvents The control of hormone liberation is in part internal through a diurnally rhythmic mechanism, and partly reflex involving stimulation of the compound eyes, with the relative importance of the two varying with the species

IV Hormones and Retinal Pigment Movements

A Retinal Pigments and Their Normal Activities

The principal photoreceptors of the higher crustaceans are the compound eyes each of which is composed of a relatively large number of units the ommatidia The determination of the manner in which these eyes function and the physical adaptation of the eyes to changes in light intensity are both affected by the movements of pigments within certain cellular elements within the eyes This subject has been reviewed by Parker (113) The pigments participating in these functions in crustaceans fall into three groups (1) the distal retinal pigment (2) the proximal retinal pigment and (3) the reflecting pigment (Fig 6)

The distal retinal pigment is the black pigment, melanin This pigment occupies two cells which surround the distal portion of each rodlike ommatidium to form a light absorbing, sleevelike casing In bright light the pigmented sleeve elongates and encases the whole length of the dioptric portion of the ommatidium, effectively providing that all light

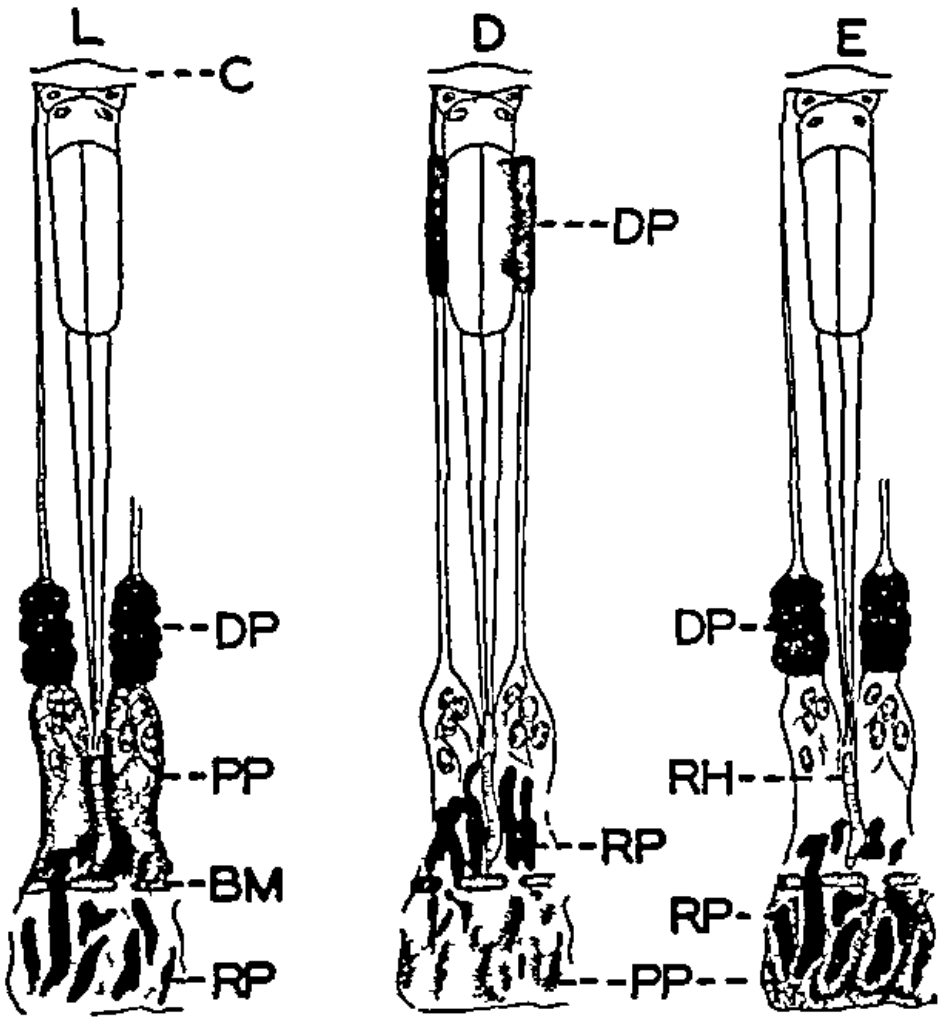


FIG 6 —Ommatidia from the eyes of *Palaemonetes vulgaris* in light (L), dark (D) and in dark following injection of extract of eyestalks from light-adapted specimens (E) C, cornea, DP, distal pigment PP, proximal pigment BM, basement membrane RP, reflecting pigment RH, rhabdome (From Kleinholz 77)

which passes through an eye facet of a given ommatidium remains within that particular one Thus, in bright light the eye functions as a mosaic type with only the light entering an ommatidium finally stimulating the sensory elements of that ommatidium In darkness or in very low light intensity the pigmented sleeve is reduced in length and surrounds only the distal region of the dioptric apparatus of the eye This condition allows light to pass abundantly from the refractive apparatus of one ommatid-

ium to other neighboring ommatidia In this condition the refractive bodies of several adjacent ommatidia may cooperate to bring more light to bear upon the sensory portion of a single one The small amount of light may thus be used more efficiently In these roles the distal retinal pigment cells are supported by the activity of the proximal retinal pigment cells which also contain melanin

The proximal retinal pigment migrates within the retinula cells In bright light the pigment spreads throughout the retinula cells to form an elongated collar surrounding the central receptive rhabdomes, effectively preventing the passage of light from one rhabdome to neighboring ones In maximally light adapted eyes, the distal retinal pigment together with the proximal may form almost a continuous sheath of pigment extending the whole length of the ommatidium In darkness the proximal pigment migrates proximally even to a point beneath the basement membrane

The third pigment is the white reflecting pigment, guanine, which comprises the tapetum of the eye This granular pigment in bright light typically migrates proximally to a position beneath the basement membrane, while in very low light intensity or in darkness it moves distally to surround the rhabdomes, where it is believed to function to increase the stimulative efficiency of the weak light entering the eye by reflecting any light which strikes it back over the receptive elements

The three pigments typically respond to light intensity changes as have just been described but the responses are often complicated by the possession by the animal of a diurnally rhythmic activity of the retinal pigments with one or more of the three pigments exhibiting, independently of light intensity changes, movements to the dark- and light adapted conditions during nighttime and daytime, respectively (15,78,79,145,147,148)

B The Role of Hormones

Bennitt (13,14) was the first investigator to suggest that hormones might be involved in the control of the movements of retinal pigments *Bennitt's experiments consisted of stimulating one eye of crustaceans of* several species and observing the effect of this stimulation upon the contralateral eye maintained in darkness He noted that the shielded eye also tended to assume the light-adapted condition This removed the possibility of the retinal responses being exclusively that of independent effectors but did not permit any decision as to whether the control was through nervous innervation or through blood borne hormones Bennitt favored the hormonal alternative in view of the apparent absence of any histological evidence of innervation of the active, distal retinal

pigment cells An endocrine interpretation was supported by the observations of Welsh (146) that dark-adapted Palaemonetes subjected to light for twenty minutes would rapidly commence retinal light adaptation through appropriate migrations of their pigments This change for the distal retinal pigment continued for many minutes after the animals were returned to darkness This fact appeared to find its most reasonable explanation in terms of the continued activity of a light-adapting hormone which persisted in the blood for some time after the stimulus inducing its discharge had ceased

The first direct evidence in support of a hormonal hypothesis of control of crustacean retinal pigments was provided by Kleinholz (76,77), who noted that when aqueous extracts of eyestalks of light adapted Palaemonetes were injected into dark-adapted animals kept in darkness, the latter became light-adapted with respect to their distal and reflecting retinal pigments (Fig 6) The proximal pigment showed no response With doses containing the equivalent of one to three eyestalks, the rate of the light adaptation was very similar to that normally induced by light Support for the assumption that the eyestalks contained the source of a hormone normally involved in this role came in the observation that eyestalks of dark-adapted specimens were significantly less effective Muscle extracts, or physiological salt solutions by themselves, had no effect Injection of fully light-adapted Palaemonetes with eyestalk extract produced no changes The eyestalks of a number of other species of crustaceans (Cancer, Libinia, Uca, Callinectes, and Carcinides), all brachyurans were extracted and these extracts assayed upon dark-adapted Palaemonetes in darkness All the extracts except those of Callinectes showed strong light-adapting activity on distal retinal pigment, Callinectes extracts gave only weak responses

The activity of eyestalk extract upon retinal pigment migration was confirmed by Welsh (151) working upon Cambarus Welsh found that boiled extracts were fully effective, and that the response obtained upon injection of Cambarus eyestalk extract into Cambarus varied with the dosage With doses containing about one quarter of an eyestalk, only the distal retinal pigment responded, but with doses equivalent to about two eyestalks both the distal and proximal pigments responded It will be recalled that Kleinholz had found no response of the latter pigment of Palaemonetes to the injections of Palaemonetes eyestalk extract On the basis of his experiments Welsh believed that both of the pigment cell types were under control of a single hormone produced in the eyestalks with the two pigments differing in their threshold of response Attempts to locate the specific source in the eyestalk of the principle involved led Welsh (153), still working on Cambarus, to find that the sinus gland was

the most effective tissue Some activity was also found in the medulla terminalis but this he believed was due to residual sinus gland tissue or to hormonal material that had escaped from the gland The supra-esophageal ganglion showed no activity It thus appears that the crustacean sinus gland is the source of a principle which is at least partly responsible for the light adapted condition of the two, dark, retinal pigments of Cambarus, and, in all probability, also of the distal and reflecting pigments of Palaemonetes investigated by Kleinholz (77)

There is no evidence indicating that more than one retinal pigment hormone is operative in the crustaceans There has been no suggestion in the literature that retinal pigments exhibit any degree of independence of activity with respect to one another In fact, there would appear to be no functional usefulness of such an independence

Among the numerous crustaceans in which diurnally rhythmic retinal pigment movements in constant illumination have been described, it is not possible to arrange any constant series of relative responsiveness of the three pigment types to a single hormone which would account for all the observations One may establish a hypothesis that the three pigments of the eyes are controlled in each species by one hormone, each pigment showing its individual threshold of response to this hormone However, on the basis of this hypothesis it would be necessary to assume that each species showed either its characteristic pigmentary response pattern to a single hormone common to all species, or that the retinal pigment hormone differs somewhat from species to species At present there is not sufficient evidence to permit us to choose between these alternatives Furthermore, the possibility of an action of a second, antagonistic hormone is not yet ruled out The problem is still further complicated by the strong suggestion that other factors than hormones operate in the control of retinal pigments Evidence for such other factors is found in the responses of eyes deprived of circulation to changes in light intensity (13) the total or partial independence of the two eyes of an animal (13 14 42,111), and the differential response of the dorsal and ventral regions of a single retina to a black background (84)

Welsh (153) assumed that the observed diurnally rhythmic movements of the retinal pigments of Cambarus was directly due to the periodic liberation of a sinus gland hormone the gland being in turn supplied by an inhibitory nerve The latter view was supported by observations that depressants of nervous activity such as low temperature (153), oxygen deficiency (16) and anesthesia (13 146,153) give rise to the light-adapted condition

At present relatively little is known of the properties of the retinal pigment hormone (RPH) of the eyestalk Kleinholz (77) considered that

the hormone was probably identical with the chromatophorotropic one influencing the dark chromatophores of the body, with all the responses explainable in terms of different thresholds of the various pigmentary cells Hanstrom (60) and Abramowitz (4) believed that these two principles could not be identical since the body chromatophores could assume any state regardless of whether the retinal pigment was in either the dark- or light-adapted state Kleinholz (80) adopted this same view after finding that it required approximately twenty times the dosage of eyestalk extract to render the eye light adapted as to lighten the bodies of shrimp, hence with a single hormone it would not be possible to account for the very commonly observed phenomenon of a dark bodied shrimp with a light adapted retina Therefore it now seems improbable that the retinal pigment hormone is identical with any of those normally responsible for the color changes within the animals although there is the possibility the retinal pigment hormone may exert some influence upon chromatophores

C General Summary

The evidence at hand strongly suggests that a sinus gland hormone, RPH, is normally cooperating with other factors in crustaceans to determine the state of the retinal pigments within the eye Final resolution of this question awaits study of the effects of removal of the sinus glands without damage to the retinal elements The evidence at hand does not favor the possibility that such a hormone is identical with any of the principal ones normally controlling color changes of the body in the animals

V Hormones and Molting and Growth

A The Molting Process

The Crustacea comprise one of a number of animal groups whose bodies are encased in a relatively rigid external covering and whose growth is dependent upon periodic casting off of the old skeleton and the formation of a new one Thus, such animals grow discontinuously, the total growth being restricted to very brief intervals immediately following the loss of the old skeleton (molt or ecdysis), with relatively long intervening periods (intermolt) in which no change in size can occur The molt proper in crustaceans is usually preceded by a premolt period in which calcium is resorbed from the exoskeleton and deposited in certain organs of the body, such as gastroliths in the wall of the stomach of the crayfish (72,96,123,130), or in the hepatopancreas (115 122) The premolt period is also a time of increase in water content of the body which reaches a maximum immediately following molt (11,46,108 115,122 130) The increase in size of the animal at the time of molt seems to result

almost exclusively from water uptake, whereupon the new skeleton is hardened by calcium salts contributed from the storage depots and from additional calcium absorbed directly from the external environment (67,83,102,115) There is a gradual increase in oxygen consumption in crayfish in premolt (128)

The frequency with which normal animals molt varies with a number of factors, including age, food supply, and species Young crustaceans in their first year of life usually molt many times Young crayfish have been found to molt every two weeks or so (136) Older specimens molt less frequently, the molt typically being a seasonal phenomenon Adult crayfish normally molt twice a year, once in April or May, and a second time in July or August (139,142)

B The Role of Hormones

In the course of studies of the role of the eyes and the eyestalks in the control of color changes in crustaceans (see Section III, C above) several investigators observed that animals from which the eyestalks had been removed molted more frequently than normal ones (8,65,103) These observations were capable of interpretation in any one of three ways First, operative injury by itself might accelerate molts Darby (45) reported accelerated molting in injured *Crangon armillatus* Secondly, it was possible that nerve centers in the eyestalk controlling molt were being removed by the operation Thirdly, it was possible that the eyestalks contained the source of a molt-inhibiting hormone

Brown and Cunningham in 1939 (31) reported that adult Cambarus approaching the spring molt molted significantly sooner in those specimens from which the eyestalks had been removed than in the normal ones Removal of a single eyestalk also resulted in an earlier molt, but less significantly so Eyestalkless animals into which sinus glands were implanted into the ventral abdominal sinus showed their molt retarded even beyond the time seen for normal animals Finally, implants of eyestalk tissue from which the sinus glands had been carefully removed showed no significant modification of the time of molt These investigators concluded that molt acceleration in eyestalkless animals was a result of the removal of the sinus glands which normally produced a molt-inhibiting hormone

The following year Smith (136) working upon very young specimens of another species of Cambarus confirmed the molt-accelerating effect of eyestalk removal He noted that the intermolt period in young animals was normally about twelve days, but that after removal of the eyestalks this interval was shortened to approximately eight days Smith ruled out the possibility that operative injury alone was responsible for the

shortened intermolt periods by discovering that animals subjected to a more severe operation, namely retina removal, actually showed a lengthened intermolt period of about fourteen days Abramowitz and Abramowitz (10) and Kleinholz and Bourquin (82) demonstrated accelerated onset of the first molt and shortened intermolt periods for the fiddler crab, Uca None of the later workers followed up their eyestalk removal experiments with experiments involving eyestalk tissue or sinus gland replacement, therefore contributing no evidence to differentiate between a hormonal and nervous interpretation of their results Smith, and Abramowitz and Abramowitz, favored an interpretation in terms of an eyestalk-originating hormone whose action was to inhibit molt, Kleinholz

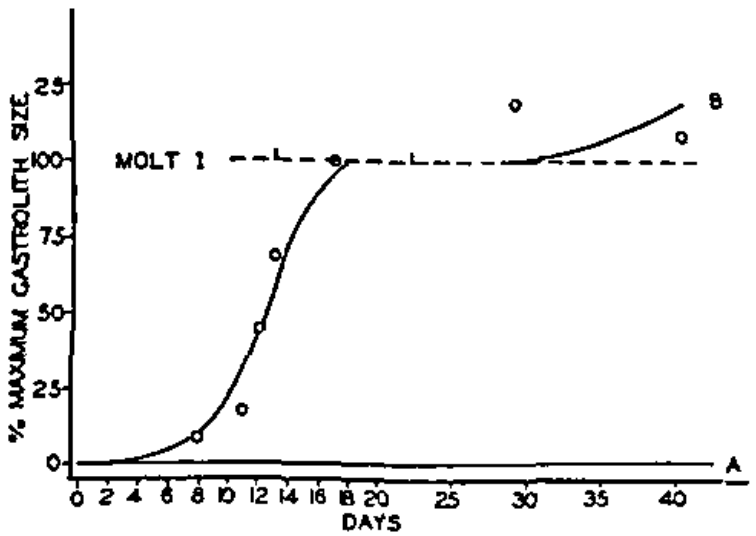


FIG 7 —Curve B shows rate of gastrolith formation following eyestalk removal in *Cambarus immunis* Curve A shows inhibition of gastrolith formation in eyestalkless specimens following weekly sinus gland implants (From Scudamore, 130)

and Bourquin tended to favor an interpretation in terms of a nervous center removal

The hormonal interpretation of the relation of eyestalks to molting was given decisive support by a study of the relation of the eyestalks and sinus glands to gastrolith deposition in the wall of the crayfish stomach by Kyer (96) and Scudamore (130) These investigators independently discovered that gastrolith formation could be induced very effectively by removal of the eyestalks (Fig 7) Removal of the sinus glands by themselves also resulted in gastrolith formation (28) Both Kyer and Scudamore found that gastroliths had already commenced to form within 24 hours following eyestalk amputation These bodies increased in size, first relatively slowly, then after eight to ten days at a rapidly accelerating rate, terminated by molt usually between fifteen and twenty days follow

ing the operation The formation of gastroliths could be entirely prevented by periodic implants of sinus glands into the abdominal region of the eyestalkless animals No other tissue of the eyestalk showed any significant inhibition of gastrolith formation Scudamore found that sinus gland implants remained active in inhibiting gastrolith formation and molt for about a week Each molt was followed directly by another premolt period, contrary to the case in normal animals

Very strong support for a normal molt control function of the sinus gland was presented by Pyle (120), who described both acidophilic and basophilic inclusions within the cells of the sinus gland There was found to be a cyclic change in relative abundance of the two types of inclusions in Cambarus which corresponded with the molt cycle In the premolt period there was a preponderance of the acidophilic substance, in the immediate postmolt period, basophilic material was predominant

The changes induced in Cambarus by eyestalk removal resembled very closely the changes observed in a normal premolt period In addition to the formation of gastroliths there was simultaneously a gradual uptake of water and an increase in O_2 consumption during the period between the operation and the actual molt, and these changes were also either greatly reduced or abolished as a result of abdominal, sinus gland implants (130) It therefore seemed reasonable to suppose that all these changes were simply part of a total molting mechanism which was inhibited by a single sinus gland hormone Another process proceeding during the premolt period is the resorption of inorganic salts from the old exoskeleton Koller (92), working on the mechanism of action of eyestalk hormone on Crago dark chromatophores, was led to suspect that its action involved calcium ions He made the observation that the exuvia of eyestalkless shrimp contained less inorganic material soluble in HCl than those of normal shrimp This observation was confirmed by Plankemann (118) working upon crayfish, but denied by Kleinholz and Bourquin (83) for Palaemonetes These observations of Koller and of Plankemann do not necessarily require for their interpretation any other hormone than a molt inhibiting one At the time of their work there was no good evidence of a molt control factor from the eyestalk The total absence of the sinus glands following eyestalk amputation might well result in more complete reduction in skeletal inorganic material than that seen in a normal suppression of gland activity typically associated with the molt

Assuming that the influence of the eyestalk upon the inorganic salt content of cast exoskeletons is the result of variations in quantity of the molt inhibitor, Koller's (92) work gives us some reason to suspect that the molting hormone is not identical with the eyestalk principle concerned with control of the dark chromatophores He could observe no signifi-

cant difference in the calcium contents of the exuvia from animals kept on black backgrounds, and on white ones

Growth is normally associated with molt in crustaceans Several investigators have observed that eyestalkless specimens become larger than normal ones Abramowitz and Abramowitz (10) noted some relatively huge specimens among their eyestalkless Uca surviving at the end of a 48-day experiment They were inclined to interpret this in terms of the induction of additional molts Smith (136) noted that eyestalkless young Cambarus ate a great deal and became larger than normal specimens in the same age group, which also had molted less frequently Scudamore (130) working upon adult Cambarus confirmed the larger food consumption in eyestalkless forms and noted that thirteen crayfish induced to molt in winter by eyestalk removal showed a greater average increment of carapace length (5 61 $\pm$ 0 17%) than thirteen specimens in their normal spring molt (1 80 $\pm$ 0 77%), and concluded that the molt inhibitor was also a growth retarding principle It is known however, that the increment of growth at molt is significantly different for the two normal molts of crayfish each year and probably this difference is related to the nutritional state of the animal, which may well also differ from winter to spring

There is some suggestion that other factors, perhaps involving an antagonistic hormone, operate in molt control within crayfish Scudamore described the gastrolith as a laminated structure The number of layers comprising it agreed with the number of days elapsing following eyestalk removal Further analysis indicated that there was a diurnal rhythm in the deposition of material in the gastrolith, with activity proceeding principally at night Scudamore found that strong stimulation of the stubs of the eyestalks or injection of brain extract, in an eyestalkless animal resulted in a period of elevated O_2 consumption in the animal It therefore appears possible that a hormone from anterior central nervous organs might operate in acceleration of the molting process

It has been known for some years that female crustaceans carrying eggs upon their pleopods do not molt in the spring at the time the males do, but postpone their molt until after the young are liberated This phenomenon has been studied by Hess (68) for Crangon Scudamore (130) has found that egg bearing female crayfish can be induced to molt by eyestalk removal just as readily as can males, thus showing that this normal postponement of molting, so essential to survival of the species, is a function of the sinus gland

C General Summary

The crustacean sinus gland produces a hormone whose action is that of a molt inhibitor In the absence of the molt-inhibiting hormone

(MIH) molting will occur after an interval which appears to be characteristic for each species, other factors equal, and the animals will pass from one premolt period directly to another one without any significant intermolt period such as is the case with normal animals There is also some suggestion that a second hormone, not from the eyestalks, cooperates in molt control

VI. Hormones and Other Activities

A Viability

The eyestalks appear essential for the continued life of certain crustaceans Brown (23) reported that whereas unilateral eyestalk removal from Cambarus had no effect upon the survival of the animal, bilateral extirpation resulted in a very significant shortening of the life, but that this latter could be extended to a small but significant degree by implants of general eyestalk tissue These results were confirmed and extended by Brown and Cunningham (31), who found that the average period of survival of eyestalkless crayfish could be extended from approximately one week to nearly three weeks by implantation of sinus glands by themselves, but that implantations of the remainder of the eyestalk tissue were less than half as effective These investigators concluded that a hormone produced in the sinus gland was essential to normal viability This work was confirmed by Brown (25) using the shrimp, Palaemonetes, and obtaining sinus glands for implantation from the crab, Carcinides, indicating that the principle involved was widespread among crustaceans and relatively nonspecific

This viability effect was seen in young crayfish by Smith (136), who found an average survival time of about two and a half weeks after removal of the eyestalks It was also found in *Uca pugilator* by Abramowitz and Abramowitz (10), who found that about 89% of their eyestalkless animals died during a 48-day experiment, during the same period only 16 6% of the control animals died Kleinholz and Bourquin (82), also using Uca, failed to confirm the latter work and denied that the presence of the eyestalks favored survival Scudamore (130), working on adult Cambarus during a nonmolting season, found that eyestalkless animals survived for an average of seventeen days, or about the extent of a typical premolt period while similar eyestalkless specimens, given a sinus gland implant about twice a week, survived an average of more than 38 days

All investigators who have noted a decreased viability following bilateral eyestalk extirpation have also noted that the great majority of deaths occur either during the actual molting process, or approximately at a time when a molt following eyestalk removal would be expected

This might lead one to believe that there is some actual causal connection between the death of an animal and an inability to escape from its old exoskeleton The life-prolonging action of sinus gland implants might then be interpreted in terms of postponement of this critical operation This explanation, however, obviously cannot be the whole one since, under otherwise entirely similar conditions, a large fraction of the normal animals survive molt, while a large fraction of the eyestalkless animals fail to do so Of the small fraction of the eyestalkless animals surviving one molt, only a few of these survive a second, and practically none get through a third In short, the evidence at hand clearly indicates that eyestalkless animals are less able to carry out a successful molt than are normal ones Even with frequent sinus gland implants inhibiting molting phenomena crayfish appear to survive an average of only about six weeks (130) It is quite possible, however, that the immediate cause of the deaths is a mechanical one associated with the molt proper, with the repetitive molting, following eyestalk removal, subjecting the animal to a series of very rigorous tests Animals becoming progressively weakened as a result of a second deficiency would conceivably become progressively less able to undergo succeeding molts The viability factor may not be a product of the sinus glands but may be a product of the X-organs of the eyestalk described by Hanstrom (see p 172 and references cited there) and for which no function has as yet been determined, or the effect may even be due to loss of an essential nerve center in the eyestalk

B Heart Rate

Welsh (149) observed that, when hearts of Cambarus were exposed, a significant acceleration of the heart rate could be observed following perfusion of the heart with eyestalk extract from the same species or from Palaemonetes The same investigator (152) noted that an injection of eyestalk extract into Leander both accelerated the heart beat and concentrated the red pigment of the body An alcohol extract of eyestalks, however, concentrated the red pigment but had no action on the heart, therefore Welsh concluded that two different principles were involved and believed the factor accelerating heart beat was acetylcholine Acetylcholine appears to have no significant action on the red pigment Scudamore (129) working on the closely allied shrimp, Palaemonetes, noted an extraordinarily close inverse correlation between the degree of dispersion of the red pigment and the frequency of heart beat in the animal This correlation obtained irrespective of whether the red chromatophores were responding to color of background, to light and darkness, or to injections of eyestalk extracts Furthermore, an alcohol extract of sinus glands of Carcinides was as effective as a sea water extract Scudamore concluded

that the chromatophorotropic principle influencing Palaemonetes red pigment (PLH) was also a heart accelerating one

C Blood Sugar

Abramowitz, Hisaw, and Papandrea (9) have reported that a powerful diabetogenic factor was present in the eyestalks of Uca and Callinectes Injection of extracts containing the equivalent of one Uca eyestalk into Callinectes resulted in a transitory elevation of blood sugar level from 20 mg % to a high point of about 80 mg % With smaller doses less elevation was seen Practically all the activity of the eyestalks was found to reside in the sinus glands The extracts could be boiled for several minutes without perceptible loss or gain in activity Eyestalk removal did not result in a hypoglycemia as one might expect were the sinus glands normally of great importance in this capacity

D Locomotor Activities

There have been numerous observations of a diurnal rhythm in locomotor, as well as other, activities of animals This subject has been reviewed by Welsh (150) and Park (110) Attention has recently been called to a correlation, observed by earlier workers, between the state of certain chromatophores and locomotor activity, and suggestion has been advanced that the diurnal rhythms in activity which continue under constant environmental conditions might be the result of a diurnally rhythmic secretion of a hormone or hormones

Kalmus (73), Roberts (121), and Schallek (126) have reported a loss of the diurnal rhythm in locomotor activity of crayfish following eyestalk removal but these three investigators differed greatly in the analysis of their results Kalmus found that aqueous or alcohol extracts of eyestalks injected into eyestalkless crayfish, Potamobius resulted in greatly increased locomotor activity for several hours Roberts found, on the contrary, decreased activity following injection of eyestalk extract into eyestalkless Cambarus Schallek found no influence of either sinus gland extracts or implants in Cambarus, and, since severing the optic nerves appeared to result in the same long-lasting increase in locomotory activity as eyestalk amputation, Schallek concluded that eyestalks contained an inhibitory nervous center for locomotory activity

In brief, there seems to be no clear picture at present as to what role, if any, hormones play in influencing the amount of general locomotor activity

E Ovarian Development

The sinus gland of the shrimp, *Leander serratus*, has recently been shown by Panouse (109a,109b) to possess a principle which inhibits the

development of the ovaries in this species Removal of the eyestalks in a nonbreeding season (September and October) is followed by a rapid increase in weight of the ovaries (Fig 8), the latter increasing more than seventyfold in a month and a half while unoperated controls show almost no increase (109a) Removal of the sinus glands by themselves produces a similar type of response though not quite as marked probably due to

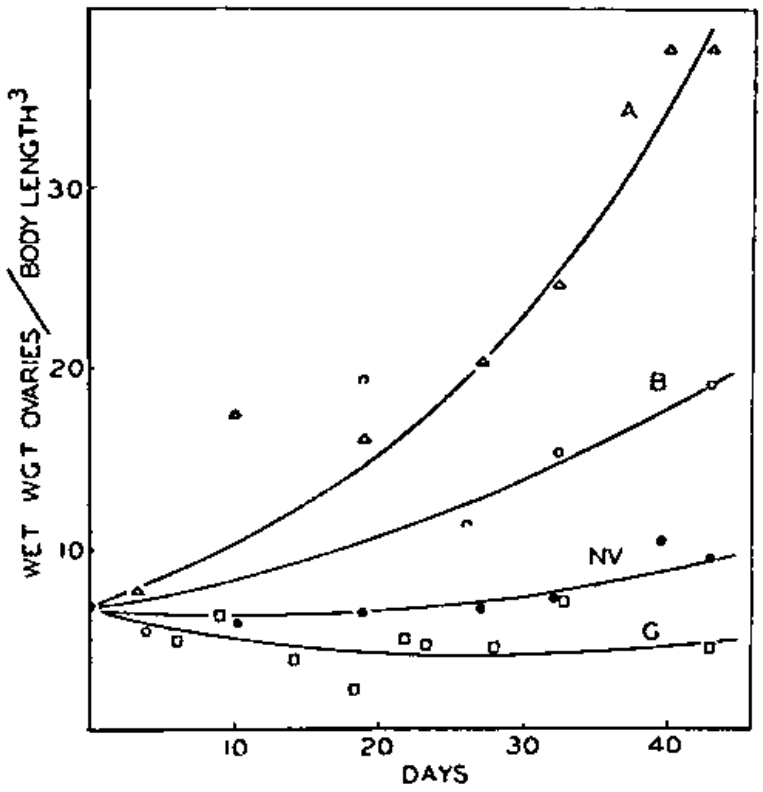


FIG 8 —Relation between the ratio $\frac{\text{ovary wet wt}}{\text{body length}}$ 3, and time (days) for Leander in a nonbreeding season A, eyestalkless B sinus glandless NV, Normal controls G, eyestalkless shrimp with a sinus gland implant (Redrawn from Panouse, 109b)

the difficulty of removing the glands in their entirety Eyestalkless specimens often even ovulate, producing apparently normal, but unseasonable, eggs at the end of the period of ovarian enlargement When, however, sinus glands are implanted at eight-day intervals, into the abdomens of eyestalkless specimens the ovarian development is inhibited (109b), the ovaries showing even less size increase than in unoperated controls

REFERENCES

1 Abramowitz, A A *Proc Natl Acad Sci U S* 21, 677–681 (1935)
2 Abramowitz, A A *ibid* 22, 521–523 (1936)
3 Abramowitz, A A *Biol Bull* 72, 344–365 (1937)
4 Abramowitz, A A *J Exptl Zoöl* 76, 407 422 (1937)
5 Abramowitz A A. *Physiol Zool* 11, 299–311 (1938)

6 Abramowitz, A A *Tabulae Biol* **161**, 267–337 (1939)
7 Abramowitz, A A *J Biol Chem* **132**, 501–506 (1940)
8 Abramowitz, A A, and Abramowitz, R K *Biol Bull* **74**, 278–296 (1938)
9 Abramowitz, A A, Hisaw, F L, and Papandrea, D N *ibid* **86**, 1–5 (1944)
10 Abramowitz, R K, and Abramowitz, A A *ibid* **78**, 179–188 (1940)
11 Baumberger, J P, and Olmsted, J M D *Physiol Zoöl* **1**, 531–544 (1928)
12 Beauvallet, M, and Veil, C *Compt rend soc biol* **117**, 688–690 (1934)
13 Bennitt, R *J Exptl Zoöl* **40**, 381–435, (1924)
14 Bennitt, R *Physiol Zoöl* **5**, 49–64 (1932)
15 Bennitt, R *ibid* **5**, 65–69 (1932)
16 Bennitt, R, and Merrick, A D *Biol Bull* **62**, 168–177 (1932)
17 Biedl, A Innere Sekretion, 2 Aufl Berlin u Wien, 1913
18 Borang S *Arkiv Zool* **25**, (19), 1–16 (1933)
19 Bottger, G *Z vergleich Physiol* **21**, 415–428 (1934)
20 Brinkman, A *Bergens Mus Skr*, No 18, pp 1–111 (1936)
21 Brown, F A, Jr *Proc Natl Acad Sci U S* **19**, 327–329 (1933)
22 Brown, F A, Jr *J Exptl Zool* **71**, 1–15 (1935)
23 Brown, F A, Jr *Proc Natl Acad Sci U S* **24**, 551–555 (1938)
24 Brown, F A, Jr *Am Naturalist* **73**, 247–255 (1939)
25 Brown, F A, Jr *Anat Record Suppl* **75**, 129 (1939)
26 Brown, F A, Jr *Physiol Zool* **13**, 343–355 (1940)
27 Brown, F A, Jr *Trans Illinois State Acad Sci* **34**, 24–28 (1941)
28 Brown, F A, Jr *Proc Soc Exptl Biol Med* **50**, 295–297 (1942)
29 Brown, F A, Jr *Physiol Zoöl* **19**, 215–223 (1946)
30 Brown, F A, Jr *Quart Rev Biol* **19**, 32–46, 118–143 (1944)
31 Brown, F A, Jr, and Cunningham, O *Biol Bull* **77**, 104–114 (1939)
32 Brown F A, Jr, and Ederstrom, H E *J Exptl Zool* **85**, 53–69 (1940)
33 Brown, F A, Jr, Ederstrom, H E, and Scudamore, H H *Anat Record Suppl* **75**, 129–130 (1939)
33a Brown, F A, Jr, and Klotz, I M *Proc Soc Exptl Biol Med* **64**, 310–313 (1947)
34 Brown, F A, Jr, and Meglitsch, A *Biol Bull* **79**, 409–418 (1940)
35 Brown, F A, Jr, and Saigh, Lorraine *ibid* **91**, 170–180 (1946)
36 Brown F A, Jr, and Scudamore, H H *J Cellular Comp Physiol* **16**, 103–119 (1940)
37 Brown, F A, Jr, and Wulff, V J *ibid* **18**, 339–353 (1941)
38 Callan, H G *J Exptl Biol* **17**, 168–179 (1940)
39 Carlson S P *Proc Natl Acad Sci U.S* **21**, 549–551 (1935)
40 Carlson S P *Kgl fysiogr Sallsk Lund Forhandl* **6**, 63–80 (1936)
41 Carstam S P *Z vergleich Physiol* **29**, 433–472 (1941)
42 Castle, E S *Proc Natl Acad Sci U S* **13**, 637–639 (1927)
43 Courrier, R *Compt rend* **173**, 668–671 (1921)
44 Cuenot, L *Arch Zool exptl*, Ser 4, **3**, 1–15 (1905)
45 Darby, H H *Anat Record Suppl* **72**, 78 (1938)
46 Drach, P *Ann inst Océanograph Monaco* **19**, 103–391 (1939)
47 Fasten N *J Morphol* **25**, 587–649 (1914)
48 Fuchs, R R *Winterstein Handbuch vergl Physiol* **3**, 1189–1656 (1914)
49 Gamble, F, and Keeble, F *Quart J Microscop Sci* **43**, 589–698 (1900)
50 Giard, A *Compt rend* **103**, 84–86 (1886)

51 Goldschmidt, R Die sexuellen Zwischenstufen Berlin, 1931
52 Haemmerli-Boveri, V *Z vergleich Physiol* **4**, 668–698 (1926)
53 Hanström, B *Z Morphol Okol Tiere* **23**, 80–236 (1931)
54 Hanstrom, B *Zool Jahrb, Abt Anat Ontog Tiere* **56**, 387–520 (1933)
55 Hanström, B *ibid* **58**, 101–144 (1934)
56 Hanström, B *Arkiv Zool* **26**, (24), 1–66 (1934)
57 Hanström, B *Psychiatr Neurol Bladen, Jaarg* **38**, 405–425 (1934)
58 Hanström, B *Kgl fysiogr Sällsk Lund Forh* **5**, 1–14 (1934)
59 Hanström, B *Proc Natl Acad Sci U.S* **21**, 584–585 (1935)
60 Hanström, B *Kgl Svenska Vetenskap Handl* **16**, (3), 1–99 (1937)
61 Hanström, B *Kgl fysiogr Sällsk Handl* **47** (8), 1–10 (1937)
62 Hanström, B *Ergeb Biol* **14**, 143–224 (1937)
63 Hanström, B *Kgl fysiogr Sällsk Handl* **49** (11), 1–10 (1938)
64 Hanström, B *ibid* **49** (16), 1–17 (1938)
65 Hanström, B Hormones in Invertebrates Oxford Univ Press London 1939
66 Hanstrom, B *Kgl fysiogr Sällsk Handl* **52** (4), 1–19 (1941)
67 Hecht, S *Science* **39**, 109–109 (1914)
68 Hess, W N *Biol Bull* **81**, 215–220 (1941)
69 Hitchcock, H B *ibid* **80**, 26–30 (1941)
70 Hosoi, T *Faculty Sci Imp Univ Tokyo* **3**, 265–270 (1934)
71 Hughes, T E *J Exptl Biol* **17**, 331–336 (1940)
72 Huxley, T H The Crayfish Paul Trench, Trubner & Co London, 1906
73 Kalmus, H *Z vergleich Physiol* **25**, 798–802 (1938)
74 Keeble, F, and Gamble, F *Proc. Roy Soc London* **B35**, 461–468 (1900)
75 Keeble, F, and Gamble, F *Trans Roy Soc London* **B196**, 295–388 (1904)
76 Kleinholz, L H *Proc Natl Acad Sci U S* **20**, 659–661 (1934)
77 Kleinholz, L H *Biol Bull* **70**, 159–184 (1936)
78 Kleinholz, L H *ibid* **72**, 24–36 (1937)
79 Kleinholz, L H *ibid* **72**, 176–189 (1937)
80 Kleinholz, L H *ibid* **75**, 510–532 (1938)
81 Kleinholz, L H *Biol Rev Cambridge Phil Soc* **17**, 91–119 (1942)
82 Kleinholz, L H, and Bourquin, E *Proc Natl Acad Sci U S* **27**, 145–149 (1941)
83 Kleinholz, L. H, and Bourquin, E *J Cellular Comp Physiol* **18**, 101–107 (1941)
84 Kleinholz, L H, and Knowles, F G W *Biol Bull* **75**, 266–273 (1938)
85 Kleinholz, L H, and Welsh, J H *Nature* **140**, 851 (1937)
86 Knowles, F G W *Pubbli Staz Napoli* **17**, 174–182 (1939)
87 Knowles, F G W, and Callan, H G *J Exptl Biol* **17**, 262–266 (1940)
88 Koller, G *Verhandl deutsch zool Ges* **30**, 128–132 (1925)
89 Koller, G *Z vergleich Physiol* **5**, 191–246 (1927)
90 Koller, G *ibid* **8**, 601–612 (1928)
91 Koller, G *Biol Revs* **4**, 269–306 (1929)
92 Koller, G *Z vergleich Physiol* **12**, 632–667 (1930)
93 Koller, G Hormone bei wirbellosen Tieren Akadem Verlagsgesellschaft, Leipzig, 1938
94 Kollman, M *Ann Soc Nat, Paris*, Ser 9, **8**, 1–238 (1908)
95 Kropp, B, and Perkins, E B *Biol Bull* **64**, 28–32 (1933)
96 Kyer, D L *ibid* **82**, 68–78 (1942)

97 Lelu, P Les Correlations Humorales chez les Invertebrates Gauthier-Villars, Paris, 1938
98 Le Roux, M L *Compt rend soc biol* **192**, 889–891 (1931)
99 Le Roux, M L *ibid* **193**, 885–887 (1931)
100 Lipschutz, A The Internal Secretions of the Sex Glands W Heffer, Cambridge, 1924
101 McVay, J A Thesis, Northwestern Univ , Evanston, Illinois, 1942
102 Maluf, N S R *J Gen Physiol* **24**, 151–167 (1940)
103 Megusar, F *Arch Entwicklungsmech Organ* **33**, 462–665 (1912)
104 Minkiewicz, R *Bull Acad Sci Cracovie* November, 918–929 (1908)
105 Miyashita, Y *Annot Zool Jap* **14**, 197–201 (1933)
106 Mori, Y *Z wiss Zool* **144**, 289–316, 573–612 (1933)
107 Okada, Y K , and Miyashita, Y *Mem Coll Sci Kyoto Imp Univ* **10**, 169–208 (1935)
108 Olmsted, J M D , and Baumberger, J P *J Morphol* **38**, 279–294 (1923)
109 Panouse, J B *Compt rend soc biol* **135**, 19–20 (1941)
109a Panouse, J *Compt rend* **217**, 553–555 (1943)
109b Panouse, J *ibid* **218**, 293–294 (1944)
110 Park, O *Ecol Monographs* **10**, 485–536 (1940)
111 Parker, G H *Bull Mus Comp Zoöl* **30**, 275–300 (1897)
112 Parker, G H *Biol Revs* **5**, 59–90 (1930)
113 Parker, G H *Frgeb Biol* **9**, 239–291 (1932)
114 Parker, G H *Proc Am Acad Arts Sci* **73**, 165–195 (1940)
115 Paul, J H , and Sharpe, J S *J Physiol* , **50**, 183–192 (1916)
116 Perkins, E B *J Exptl Zool* **50**, 71–105 (1928)
117 Perkins, E B , and Snook, T *Proc Natl Acad Sci U.S* **17**, 282–285 (1931)
118 Plankemann, H *Schr naturw Vereins Schleswig-Holstein* **21**, 195–216 (1935)
119 Potts, F A *Quart J Microscop Sci* **50**, 599–621 (1906)
120 Pyle, R W *Biol Bull* **85**, 87–102 (1943)
121 Roberts, T W *Anat Record Suppl* **81**, 46–47 (1941)
122 Robertson J D *Proc Roy Soc London* **B124**, 162–182 (1937)
123 Robertson, J D *Biol Rev* **16**, 106–133 (1941)
124 Robson, G C *Quart J Microscop Sci* **57**, 267–278 (1911)
125 Runnstrom, S *Bergens Mus Skrift* **3**, (2), 1–115 (1925)
126 Schallek, W *J Exptl Zoöl* **91**, 115–166 (1942)
127 Scharrer, B *Physiol Revs* **21**, 383–409 (1941)
128 Scudamore, H H *Anat Record Suppl* **81**, 122–123 (1941)
129 Scudamore, H H *Trans Illinois State Acad Sci* **34**, 238–240 (1941)
130 Scudamore, H H Thesis, Northwestern Univ , 1942, Abstracted in part in *Anat Record Suppl* **84**, 514–515, 515, 515–516
131 Sjogren, S *Zool Jahrb Abt Anat Ontog Tiere* **58**, 145–170 (1934)
132 Smith, G *Quart J Microscop Sci* **54**, 590–604 (1910)
133 Smith, G *ibid* **57**, 251 265 (1911)
134 Smith, G *ibid* **59**, 267–295 (1913)
135 Smith, H G *Proc Roy Soc London* **B125**, 250–263 (1938)
136 Smith, R I *Biol Bull* **79**, 145–152 (1940)
137 Stahl, F *Arkiv Zool* **30 B**, No 8 (1938)
138 Stahl, F *Kgl fysiogr Sallsk Handl* **49** (12), 1–20 (1938)
139 Tack, P I *Am Midl Nat* **25**, 420–446 (1941)
140 Tucker, B W *Quart J Microscop Sci* **74**, 1–118 (1930)

141 Turner, C L *Am Midl Nat* **16**, 863–882 (1935)
142 Van Deventer, W C *Illinois Biol Monogr* **15**, 7–57 (1937)
143 Van Oordt, G J *Zool Anz* **76**, 306–310 (1928)
144 Van Oordt, G J *ibid* **85**, 33–34 (1929)
145 Welsh, J H *Proc Natl Acad Sci U S* **16**, 386–395 (1930)
146 Welsh, J H *J Exptl Zoöl* **56**, 459–494 (1930)
147 Welsh, J H *Biol Bull* **68**, 247–252 (1935)
148 Welsh, J H *ibid* **70**, 217–227 (1936)
149 Welsh, J H *Proc Natl Acad Sci U S* **23**, 458–460 (1937)
150 Welsh, J H *Quart Rev Biol* **13**, 123–139 (1938)
151 Welsh, J H *Biol Bull* **77**, 119 125 (1939)
152 Welsh, J H *J Exptl Biol* **16**, 198–219 (1939)
153 Welsh, J H *ibid* **86**, 35–49 (1941)
154 Wense T F von der Wirkungen und Vorkommen von Hormonen bei wirbellosen Tieren J A Barth, Leipzig 1938

Chapter VI

Hormones of the Gastrointestinal Tract

By HARRY GREENGARD

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I. Introduction

The alimentary tract as an endocrine organ manifests certain features which are unlike those of the other glands of internal secretion No special cell groups have ever been identified as the source of the several autacoids whose existence has been established, yet the intestinal mucosa vies with the hypophysis in the number and diversity of physiologically active substances produced These are essentially devoid of any systemic action on the organism, affecting in general only the organs concerned with digestion Their elaboration into the blood stream is brought about by the ingestion of food, and can be artificially induced by the introduction into the alimentary canal of a variety of nonspecific agents In general, they act promptly and their effects are relatively short lived

The part played by the gastrointestinal hormones in vital processes is apparently that of regulation and coordination of the activities of the digestive glands, a role which they share with the autonomic nervous system To what extent their actions can be dispensed with is a question which at present is entirely conjectural, since they are produced throughout the entire length of the small intestine, the effects of extirpation can be obtained only by performing a complete enterectomy, a procedure which in itself engenders nutritional defects so severe as to eclipse any manifestations which might be referable to the loss of hormones

It is of interest that the first substance to be characterized as a hormone was one of the group produced by the small intestine, and that the chemical make-up of none of them is at present known In general, the concentration and separation of the various active principles have been beset with difficulties, both in regard to separation from extraneous substances and to an apparent inherent sensitivity to various procedures and chemical agents This resistance to investigation is also manifested by the hormones of the anterior lobe of the hypophysis, and, as in the case of the hypophysis, the gastrointestinal hormones are proteins or nitrogenous substances Apparently this is characteristic of all endocrine tissues derived from ectoderm or endoderm, whereas the hormones produced by tissues of mesodermal origin are sterones

In the detailed discussion which follows, the hormones of the alimentary tract will be grouped according to their sites of elaboration, and considered individually regarding their discovery, action, concentration, and practical applications

II The Upper Intestine

Four active principles have been proven conclusively to be elaborated into the blood stream by the intestinal mucosa, five more have been postulated, but conclusive evidence for their existence is at present lacking Their concentration is the highest in the upper intestine, and it steadily decreases from the jejunum on downward

A Secretin

1 *Demonstration*

The first indication that substances in the intestine were effective in stimulating the external secretion of the pancreas was obtained by Claude Bernard (14), who observed the increased secretion from a pancreatic fistula after feeding He and subsequent investigators attributed this effect to a nervous reflex mechanism The existence of a stimulus more specific than food was demonstrated in Pavlov's laboratory Bekker (13) made comparative studies there of the effect of pure water, of aqueous solutions of alkaline salts, and of water saturated with carbon dioxide, and found that water was a weak stimulant, that weak alkali not only failed to stimulate but actually inhibited, and that the carbonic acid solution was a much more potent stimulator than water Subsequently Dolinski (58) used hydrochloric instead of carbonic acid, and noted that in concentrations approximating that of the gastric juice it was a powerfully effective excitant of pancreatic flow Popielski (264) demonstrated that the action was not due to absorption of the dilute acid, since no secretion was obtained when acid was placed in the stomach or rectum, and excluded from entry into the small intestine, nor was any secretion obtained when dilute acid was injected intravenously He also (265) excluded the possibility that long reflex arcs were operative, since the acid effect persisted following bilateral vagotomy, bilateral splanchnectomy, celiac ganglionectomy, and spinal cord transection, and he attributed the effect to a short reflex involving the scattered ganglia of the pancreas, located principally in the region of the duodenum This interpretation was accepted and extended by Wertheimer and LePage (348), who noted that the response progressively diminished from the duodenum downward in the intestine, and concluded that the nervous reflex was local in nature Significantly, they also tested the effectiveness of atropine in

abolishing the secretory response, and observed that it did not do so These intimations that factors other than a nervous reflex were operative were given added impetus by the experiments of Wertheimer and LePage (349), in which the pancreatic arterial supply of one dog was joined to the general circulation of another When dilute acid was applied to the duodenum of the latter, the other dog's pancreas was activated

In this manner the foundation was laid for the momentous demonstration of Bayliss and Starling (11) that the agent involved in stimulating the pancreas was a specific substance present in the intestinal mucosa, delivered to the blood stream by the presence of acid, and thereby transported to the pancreas, where it exerted its secretory effect They showed this to be the case on the basis of a series of experiments in which they excluded as completely as possible all reflex effects by sectioning all the mesenteric nerves to a loop of jejunum and obtaining a pancreatic secretory response after the perfusion of such a loop with acid, despite the denervation, and more convincingly by stripping off the mucosa from such a loop, macerating this with dilute hydrochloric acid, and injecting the neutralized and filtered extract intravenously The result of their experiment has long been common knowledge The extract thus administered stimulated an abundant flow of pancreatic juice, which they correctly ascribed to the presence of an agent named by them "secretin," and characterized as one of a group of substances designated by them as hormones The specificity of this particular agent was substantiated by their finding that acid extracts of tissues other than the intestinal mucosa failed in effectiveness

The Bayliss and Starling discovery provided a tremendous stimulus to further studies Additional evidence of the existence of a blood borne agent was secured by Enriquez and Hallion (71), who transfused blood from a dog with an acid instilled duodenum, by Fleig (88), who injected the venous drainage from the acid-instilled duodenum of a dog, and by Matsuo (234) who prepared dogs in carotid-to jugular cross-circulation All of these workers found that application of acid to the upper intestine of the donor dog resulted in a secretory response from the pancreas of the recipient Nevertheless, general acceptance of the secretin theory did not obtain for a number of years Popielski, the chief proponent of the nervous-reflex hypothesis which his own efforts did so much to discredit, believed that all of these phenomena could be accounted for along more conservative lines He asserted (266–271) that the effect of acid in the denervated loop was still reflex, on the basis of mechanical influences acting on the remaining intact intestine and stomach, and that the effect of the intravenously injected extract was nonspecific and attributable to the presence of vasodilator substances present in all tissue extracts At

the time, the latter argument was not without merit, since the Bayliss and Starling extract contained a large amount of vasodilator material Popielski showed that extracts from various animal tissues, and also from plants, would cause the pancreas to secrete The effects of blood transfusion experiments he did not consider due to a specific agent, stating that under any circumstances the transfusion of blood from one dog to another induced a secretion of the recipient's pancreas not infrequently

The controversy was conclusively settled by subsequent decisive experiments, designed along two lines The first of these was the elimination of all possible existing connections through the nervous system This was accomplished by Ivy and Farrell (78 79,156–158), who prepared dogs with a subcutaneously transplanted portion of the pancreas which secreted after feeding or after application of acid to a Thiry loop, by Ivy, Farrell, and Lueth (165), who transplanted both a loop of jejunum and a portion of the pancreas, and obtained a secretory response to the acid stimulus applied to the transplanted loop, and by Houssay and Mollinelli (149), who anastomosed an isolated pancreas and duodenum to the vascular system of an intact dog and obtained a secretion of both pancreases in response to the presence of acid in the isolated duodenum These experiments may be regarded as crucial in their demonstration of *the exclusion of nervous reflex influences* *The second line of investigation* to establish the secretin theory was to prepare concentrates free of vasodilators, and to isolate the active principle in a chemically pure form The consummation of this, which will be treated in detail in the discussion of methods of concentration and isolation which follows, served to establish as a fact the existence of a hormone mechanism for pancreatic secretion, as well as the specificity of the site of its elaboration Thus it was shown by Drewyer and Ivy (65) that vasodilatin free extracts of various animal tissues were effective only when the tissue extracted was the small intestine (with the exception of the pyloric antrum of the stomach, which yielded a small amount)

The steps in the logical proof of a hormone mechanism for the stimulation of pancreatic secretion, as set forth by Bayliss and Starling follow

(1) Acid placed in the upper intestine stimulates pancreatic secretion

(2) Acid injected intravenously does not do so

(3) The effect of acid instillation persists after denervation

(4) Stimulation is obtained in response to intravenous injection of an acid extract of the upper intestinal mucosa

The confirmatory evidence derived from subsequent work is based on the following findings

(5) Secretion is obtained from a transplanted pancreas following instillation of acid into a transplanted loop of intestine

(6) Acid placed in the duodenum of one of a cross-circulated pair of dogs will stimulate the pancreases of both animals

(7) The hormone has been isolated in the form of a chemically pure crystalline substance

These lines of reasoning have served as the prototype in the process of demonstration of the existence of all the other proved gastrointestinal hormones

2 *Occurrence*

Bayliss and Starling (11) extracted the upper intestine of a number of vertebrates, and were in all instances able to obtain secretin, with no evidence of species specificity It has also been found present by Hallion and Lequeaux (134) in autopsy material from two newborn infants who had never ingested food, by Camus (28) in fetal guinea pigs and rabbits, and by Pringle (278) in fetal cats, indicating that the cells responsible for its formation develop with the intestine during intrauterine life

The fact that significant amounts of secretin are obtained only after acid extraction of the intestinal mucosa, and that its normal process of liberation into the blood stream is dependent on the presence of food or acid in the duodenum, led Bayliss and Starling to postulate that in the intestinal mucosa it exists as prosecretin, activated by acid to secretin, which is then absorbed It was early shown, however, that acid in the intestine is not a specific stimulus A variety of substances have been found effective, either when instilled in the gut or when employed as extracting agents These include saline solution (Delezenne and Pozerski, 48), chloral hydrate (Fallois, 75), soap, alcohol (Fleig, 87), sugar, urea, glycerol (Frouin and Lalou, 104), dilute alkalis, and phosphate buffers (Mellanby and St Huggett, 243) In addition, and most convincingly, it was observed by Wertheimer and Boulet (347) that the juice pressed from intestinal mucosa without any pretreatment is effective when injected intravenously Undoubtedly therefore the hormone is present as such in the cells of intestinal mucosa, and is merely rendered soluble and absorbable by acid or, to a lesser extent, by the other reagents mentioned above

3 *Concentration and Isolation*

So numerous have been the attempts to obtain secretin in a chemically pure form that it is not expedient to enumerate them all Hence the following discussion will be limited to those contributions representing distinct advances in the preparation of potent concentrates The original Bayliss and Starling extract was prepared as indicated above, and subsequent efforts were directed toward securing concentrates free of con

taminants, especially vasodilator substances The procedure was modified by Wertheimer and LePage (349), who introduced dilute acid into the lumen of the intestine, removed it after an interval, and found the concentration of vasodilatin to be considerably reduced Dale and Laidlaw (44) found the activity was precipitable from an acid extract of mucosa by the addition of mercuric chloride, could be eluted therefrom by precipitation of the mercury with a stream of hydrogen sulfide, and the activity separated from the resulting aqueous solution by concentration to a small volume and the addition of an excess of acetone This product lent itself to further purification by conversion to an insoluble picrate, subsequently decomposed with acidified alcohol Stepp (316,317) obtained a product which at the time was highly satisfactory by extracting acetone-dehydrated mucosa with 70% alcohol, removal of impurities from the extract by raising the alcohol concentration to 95%, and precipitation of the activity with ether from the 95% alcohol-soluble portion Weaver, Luckhardt, and Koch (345) showed that the major portion of the vasodilator substances could be removed by making an extract according to the procedure of Wertheimer and LePage and saturating this with salt The resulting flocculent precipitate was potent and of low toxicity The importance of this contribution can hardly be overemphasized, it provided a process which was feasible for the preparation of large quantities of material, and a product which served as an excellent starting point for further treatment Mellanby (238,239,241) obtained a potent concentrate by extraction of ground mucosa with absolute alcohol, conversion of the alcoholic to an aqueous extract by distillation with addition of water, and treatment with an aqueous solution of bile salt followed by weak acetic acid, which caused bile acids to be precipitated, in which process the secretin accompanied it in the adsorbed state Elution of the activity was accomplished by dissolving the precipitate in alcohol and then adding acetone and ether He found the product of this treatment to be considerably more potent than any concentrate previously characterized, and believed at the time that he had isolated the pure hormone In this he erred, and his method was criticized by Mortimer and Ivy (249) and by Still (318), who found it definitely less effective than was intimated Takacs (324,326) reported obtaining a highly potent extract by ultrafiltration of the regenerated solution from a picric acid precipitate of mucosal extract Hammarsten, Wilander, and Ågren (136) precipitated the activity from a Dale-and Laidlaw extract by adding alcohol to a concentration of 95%, dissolving the resulting precipitate in a little water, and shaking with chloroform The activity was found to be concentrated in the emulsion formed at the water-chloroform interface, which material was collected, dried, and

further concentrated by mixing with lecithin, in association with which it was soluble in absolute alcohol The activity was precipitated from such a solution with an excess of acetone

In all of the investigations cited above, based principally on trial-and-error procedures, only two really significant contributions can be designated The first of these was the original Bayliss and Starling extraction, the second, the extraction and salt precipitation procedures of Weaver, Luckhardt, and Koch The third was supplied by Ivy, Kloster, Drewyer, and Lueth (160), who extracted this salt precipitate, designated by them as "A precipitate," with 70% alcohol, removed the alcohol by evaporation, and precipitated the activity by the addition of trichloroacetic acid to a concentration of 5% The resulting precipitate, collected and dried, designated as "SI," was highly potent and free of all vasodilator contaminants In addition, it had the great advantage of being uniformly reproducible and thus served as a standard for comparison in the assay of unknown preparations, as well as a basis for experimentation on further purification Still (318) found that the activity of such a concentrate could be enhanced by solution in 90% alcohol and precipitation of impurities with brucine and pyridine This procedure was not recommended by Cunningham (42), who found it inefficient and wasteful as compared to his own process of alcohol extraction of the trichloroacetic acid precipitate followed by acetone-ether precipitation and picric acid fractionation

The isolation of secretin in the form of a crystalline compound was accomplished independently by two groups of investigators Hammarsten and collaborators (135) subjected a Dale and Laidlaw extract to further refinement with picric acid, and electrodialyzed the picrate-free aqueous solution obtained by appropriate treatment, using a continuous flow of distilled water through the cathode compartment, and collecting the efflux in an aqueous suspension of salicylic acid The resulting secretin salicylate was converted into a number of other salts, including the picrolonate, which, it transpired, was crystalline Greengard and Ivy (123) fractionated SI by dissolving in acidified 80% acetone, and adding aniline to the point of complete precipitation The supernatant fluid was found to contain the secretin, it was freed from acetone and aniline by vacuum distillation, evaporated to dryness, the residue suspended in methyl alcohol, the suspension separated from any insoluble material, and the activity precipitated with an excess of ether The product, about five times as potent as SI, was dissolved in water, the solution extracted with *n*-butyl alcohol, the dissolved butyl alcohol removed by vacuum distillation, and picrolonic acid added to the point of complete precipitation The resulting insoluble picrolonate was found

to be crystalline and, like the Hammarsten product, recrystallizable by solution in pyridine, filtration, and precipitation with an excess of ether From the crystalline picrolonate any other salt could be prepared by suspending it in water, adding the appropriate acid, and extracting with ether The free base was prepared by decomposition of the picrolonate with dilute sulfuric acid, removal of picrolonic acid, and exact neutralization with barium hydroxide None of these compounds was obtainable in crystalline form Doubilet (59) obtained confirmation of the purity and potency of secretin picrolonate prepared by this method

4 *Biological Assay*

Most early workers determined the biological activity of their secretin concentrates by the logical procedure of observing the acceleration in rate of flow of juice from a cannula inserted in the pancreatic duct The importance of securing more nearly quantitative data and of excluding the possibility of variations in individual animals was recognized by Carlson (30), who recommended having at hand a standard preparation of known potency for purposes of comparison A unit for potency was assigned by Ivy, Kloster, Lueth, and Drewyer (161), who introduced the term "threshold dose" to designate that amount of secretin which caused an increase in pancreatic flow of 10 drops (0 4 ml) in a 10 minute period over that occurring in a control 10 minute period The procedure of assay as employed by Greengard and Ivy (123) consisted of determining the response to a standard SI preparation, the threshold dose of which is about 0 25 mg in most dogs, and ascertaining that quantity of unknown which elicited the same quantity of secretion as a given weight of standard The threshold dose of the unknown may then be determined by calculating the ratio of the weight of the unknown to that of the standard and multiplying by 0 25 A record of the blood pressure of the animal is essential for the validity of an assay, to exclude the complicating factor of the possible presence of vasodilator material The Hammarsten group (352) employed a procedure in which a piece of rolled up filter paper was placed in the exposed and opened duodenum of a urethanized cat, on which mixed secretions were collected and the alkalinity was titrated The number of "units" in the preparation injected was expressed as the number of tenths of a ml of 0 1 *M* acid required to neutralize it to methyl red Naturally, pancreatic juice does not represent the sole contributor to the alkalinity of such samples, but there exist more fundamental defects which will become apparent in the discussion which follows Determinations of the relative magnitude of the Ivy unit and the Hammarsten unit were made by Greengard and Ivy (123), who performed the titrations on cat pancreatic juice obtained by direct cannula

tion of the duct They found one Ivy threshold dose to be equivalent to about twenty Hammarsten cat units

5 *Properties*

a Physical Properties The less highly purified secretin concentrates are in the form of an amorphous powder, more or less discolored, sparingly soluble in water, insoluble in organic solvents, and with an enhanced solubility in dilute acid or alkali Protein free concentrates are freely soluble in water The active principle was shown to be dialyzable by the ultrafiltration procedure of Takacs (326) and the electrodialysis studies of Hammarsten (135) Secretin is readily adsorbed to a great variety of

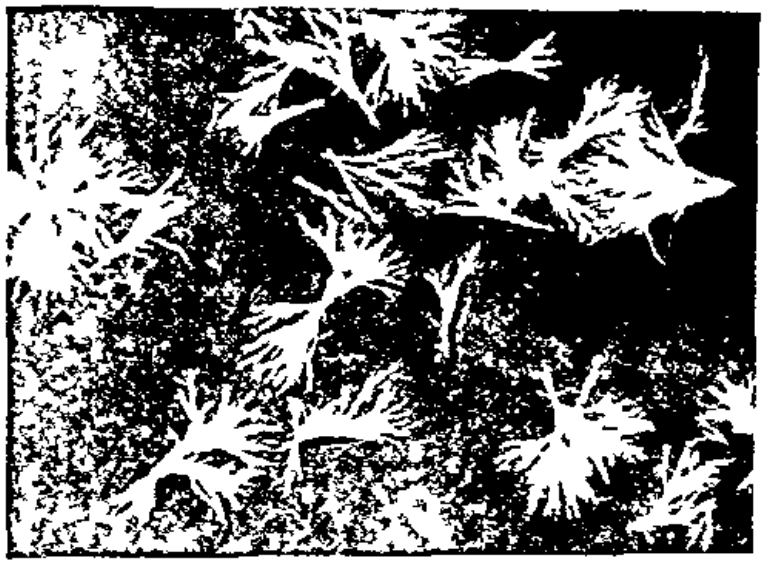


FIG 1 —Secretin picrolonate, recrystallized from pyridine (From Greengard and Ivy, 123)

insoluble substances Such adsorbates are very stable, and elution is in general effected only by decomposition or solution of the adsorbing agent

The crystalline secretin picrolonate obtained by Hammarsten had the appearance of yellow needles after pyridine-ether recrystallization An amorphous phosphate obtained from this compound manifested a molecular weight of about 5000 according to the ultracentrifuge method The Greengard and Ivy picrolonate presented the microscopical appearance of clusters of yellow needles (Fig 1), melting with decomposition at 234°–235°C The molecular weight of this material is believed to be of a relatively low order, presumptively this is evidenced by the ready diffusibility of the material, and more positively by preliminary data of actual determinations made by the freezing point and diffusion constant technique

b Chemical Properties Descriptions and analytical data on impure preparations may be entirely discounted, since they serve only to characterize the impurities present in much larger amounts than the active principle The compound is undoubtedly basic, since it is so readily amenable to acid extraction from its source, since it migrates to the cathode compartment on electrodialysis, and since it readily forms insoluble salts with picric acid and phosphotungstic acid It is a very unstable compound in solution, particularly at a pH greater than 3 0, and the decomposition is accelerated as the alkalinity is increased In solution it is also thermolabile, in acid solution it will withstand boiling for a few minutes, but in a neutral or alkaline medium it deteriorates rapidly, and autoclaving at higher temperatures engenders prompt inactivation Ågren (2) stated that the alkali inactivation was attended by only minor alterations in the structure of the molecule, since there were no changes in the optical activity or absorption spectrum of the solution He also found it to be inactivated by ultraviolet light and by hydrogen peroxide, which agencies completely altered the physicochemical properties of the solution Slow inactivation results from treatment with strong ethyl alcohol, especially on heating (161)

A number of color tests have been applied to secretin preparations The impure concentrates in general give all the reactions characteristic of proteins This is consistent with the expectation that the contaminants are largely protein in nature, but has contributed largely to the erroneous conclusion that secretin itself is a protein Both Ivy (161) and Still (318) noted the disappearance of all color tests except the biuret reaction after trichloroacetic acid purification Greengard and Ivy described the biuret reaction as bright blue for the secretin base, evidence of a basic molecule of a relatively low order of complexity Hammarsten applied the Harden and Norris (138) diacetyl reaction to secretin obtained from his crystalline picrolonate, and described it as strongly positive, indicating the prominence of a guanidine linkage The molecule itself he considered to be in the nature of a polypeptide, on the basis of the isolation of free amino acids on hydrolysis

c Composition of Pure Secretin Secretin phosphate prepared from Hammarsten's crystalline picrolonate was subjected to elementary analysis and found to contain 46% carbon, 6% hydrogen, 12% nitrogen, and 0 7% sulfur Qualitative tests for the latter element revealed that it was absent in the Greengard Ivy picrolonate Ultimate analysis of this salt showed a content of 52% carbon, 4 5% hydrogen, 20% nitrogen, and, by difference, 23 5% oxygen, these values are consistent with an empirical formula C_3H_3ON The Hammarsten product liberated about 7% of its nitrogen on treatment with nitrous acid, indicating the presence

of free amino groups Such a structure was found absent from the Greengard-Ivy product on the basis of its failure to react with acetyl chloride or methyl magnesium iodide in ether solution, which also ruled out the possible presence of any reactive hydrogen atom Hammarsten concluded on the basis of the apparent high molecular weight of their material that picrolonic acid made up only a small proportion of their picrolonate Greengard and Ivy isolated the picrolonic acid from their picrolonate and found it to make up about 80% of the compound In support of this finding, they noted that the free base isolated from the picrolonate was about five times as potent on a weight-for weight basis

The differences above cited are a clear indication of dissimilarity in the two picrolonates, that of the one group a complex compound, that of the other a relatively simple one It appears logical that the Hammarsten group isolated a crystalline secretin-protein complex, whereas Greengard and Ivy obtained secretin itself, the two bearing a relationship to each other analogous to that of thyroglobulin to thyroxin In support of such a conception is the finding of Ågren and Hammarsten (4) that digestion by aminopolypeptidase of secretin liberated from their crystalline picrolonate resulted in the liberation of ten amino acids, with no loss in secretin activity They considered these amino acids to constitute a portion of the secretin molecules, a statement which is unwarrantable

Crystalline secretin picrolonate was found by Greengard, Wolfrom, and Ness (130) to be a definitely and uniformly crystalline compound on the basis of x-ray diffraction patterns and microscopic examination It was split by extraction with warm nitroethane into soluble and insoluble fractions The former separated on cooling the nitroethane, the resulting crystals were found to be inert with respect to secretin activity, and were subsequently chemically identified as aniline picrolonate in the case of material not subjected to pyridine-ether recrystallization, and as pyridine picrolonate after such a recrystallization procedure had been applied The nitroethane insoluble fraction was an amorphous picrolonate containing all the secretin activity and not crystallizable except by re treatment with aniline or pyridine The x ray diffraction patterns of the secretin picrolonate studied were found to depend upon the solvent employed Thus, in the case of material not recrystallized from pyridine, the diffraction pattern was identical with that of aniline picrolonate, after recrystallization, it was identical with secretin picrolonate, and the biologically active nitroethane insoluble residue yielded only the pattern of the polystyrene capillary tube used as a container Thus, the active crystalline picrolonates were demonstrated to exist in the form of mixed crystals, an aniline secretin-picrolonate or a pyridine secretin picrolonate complex, depending on whether or not the material had been recrystal-

lized from pyridine It is of interest to note that the only successful efforts to crystallize secretin have involved the use of aniline or pyridine, the latter solvent was the one employed by Hammarsten

d Physiological Effects The outstanding action of secretin is a stimulation of the flow of pancreatic juice which is due undoubtedly to a direct effect on the acinar cells, as attested by the persistence of its action after denervation and transplantation An increase in the metabolic activity of the gland occurring during its action was demonstrated by Gerard and Still (107), who found a 20–50% increase in the respiratory rate of isolated pancreas from a rat after treatment with a very small quantity of the hormone, not observed when any other tissue (with the possible exception of liver) was tested, and by Kiyohira (180),

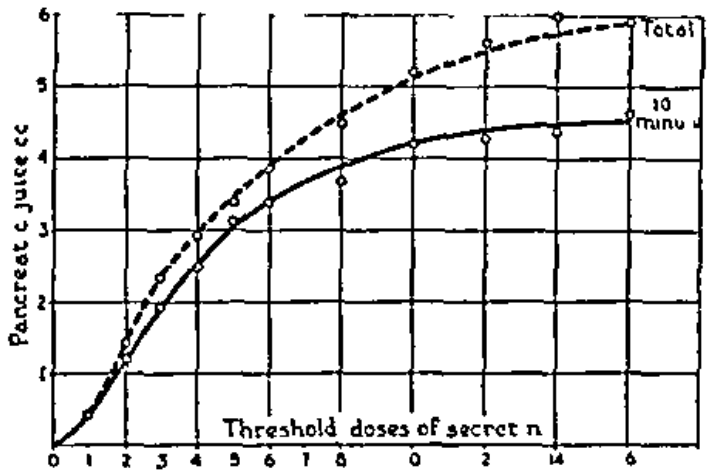


FIG 2 — Average total and ten minute volume outputs of pancreatic juice in the anesthetized dog in response to increasing doses of secretin (From Greengard *et al* 126)

who noted the oxygen uptake of pancreatic tissue in a Warburg apparatus was increased 6–18% by secretin whereas other tissues tested were unaffected

The concentration action curve of secretin was determined by Greengard, Stein, and Ivy (126) using doses ranging from subminimal to supermaximal, and plotting the volume response against dosage An S-shaped curve was obtained (Fig 2) This is in contradistinction to a report by Lagerlof (206), who found that in human subjects with a duodenal tube in place the volume response was in strikingly direct proportion to the dosage He may have used doses covering only a small limb of the total curve The former investigators also found that when secretin was injected at a constant rate in definite amounts, an injection rate corresponding to 0 0007 mg of secretin base per minute was the minimum

effective dose, whereas twenty times this quantity was required to stimulate the gland at its maximum rate Thus, assuming that when secretin is injected at a constant rate in submaximal doses a state of equilibrium is attained so that it is removed from the circulation as rapidly as injected, it follows that the quantities specified must be present in the circulation in order to excite the pancreas minimally and maximally Such an assumption is entirely in keeping with the fact that the gland attains a constant rate of secretion for any continuous dosage of secretin less than supramaximal

The effect of secretin on the composition of pancreatic juice has been shown by many investigators to be the production of a secretion low in enzymes (68,236,248,281,319,357) especially in contradistinction to the enzyme concentration following vagus activation Mellanby (240) was sufficiently impressed by such findings so that he believed secretin to control only the fluid and alkali output of the pancreas, whereas the vagus regulated the enzyme production It is now known that such a concept was entirely erroneous and that a hormonal as well as a nervous factor acts to stimulate the elaboration of the pancreatic enzymes Even in the case of pure secretin, an enzyme free secretion has never been recorded to be elicited The ratio of enzyme concentration to volume output has been noted to vary inversely with the rate of secretion Greengard, Dutton, and Ivy (118) observed that at the height of the secretin effect the enzyme concentration was minimal

As a result of numerous experiments, most of them unrecorded, it has been established that the pancreatic response to secretin is inexhaustible, and that a pancreas will manifest an undiminished response after hours of repeated injections providing the animal remains in good condition Artificially raising the body temperature of a dog was shown by Osborne and Greengard (258) to increase the response to secretin, whereas lowering the temperature had the converse effect

Several effects other than the pancreatic have been attributed to secretin, but nearly all of these have been shown by more recent work either not to exist or to be due to principles other than secretin, present in the cruder extracts One such action, however, has been shown to be manifested by the crystalline material, and is therefore truly a secretin effect namely, its action on the liver to stimulate bile formation The demonstration of this actually antedated that of the pancreatic effect Rutherford (289) first observed that acid instillation in the duodenum produced an increase in bile flow, a finding the significance of which was overlooked until Bayliss and Starling (11) obtained a similar effect from the intravenous injection of extracts of intestinal mucosa Confirmatory evidence has been obtained by a number of workers (47,62,101,146,255,

259) Exclusion of vascular influence was obtained by Lueth and Kloster (229), who used a dilatinfree secretin, and Fleig (89) obtained a cholegogue response to a transfusion of blood from the venous drainage of an acid-instilled intestinal loop Mellanby (240) considered that the cholegogue effect was due to the entry of pancreatic juice into the intestine Still (318) and Still, McBean, and Reis (320), however, noted a cholegogue effect to be manifested by depancreatized as well as normal dogs The possibility of absorption of any humoral agent from the alimentary tract was entirely ruled out by the experiments of Tanturi, Ivy, and Greengard (329), who injected purified secretin into dogs and cats from which all abdominal viscera had been extirpated except the liver and bile passages, and found the cholegogue response to persist

6 *Metabolism*

The interdigestive phase of pancreatic secretion is very slight, as demonstrated by Crittenden and Ivy (39), and after the ingestion of a meal the activity of the gland subsides rapidly as the upper intestine empties When a single injection of secretin is given, the duration of action depends on the quantity administered, but is never prolonged beyond a half hour or so It is thus apparent that secretin disappears quite promptly from the circulation The mechanism of such disappearance was studied by Greengard, Stein, and Ivy (127), who found the hormone to be quite rapidly inactivated on incubation with blood The rate of inactivation was found to depend on the temperature of incubation, the optimum being 37°C , on the pH, the effective range of which was 3 5–8 5, with an optimum of 7 4, and on the concentration The inactivating factor was present in plasma or serum, and absent from saline washed cells, and it was rendered ineffective by heating to above 60°C It was concluded on the basis of these findings that a factor is present in blood serum which destroys secretin, and which from its behavior is presumably an enzyme, designated "secretinase" Further confirmation of the enzymic nature of the agent was supplied by Doubilet (59), who found the potency of injected secretin to be enhanced by pretreatment of the animal with vitamin K, and the *in vitro* destruction of secretin by serum to be effectively inhibited by the addition of vitamin K to the mixture Such findings are readily explained on the basis of an enzyme-inhibitory action The existence of such a factor in the urine has also been noted (128) Its presence in the blood provides a satisfactory explanation for the gradual cessation of pancreatic secretion following stimulation by exogenous or endogenous secretin, as well as the failure ever to demonstrate any secretin activity in the urine Obviously, the blood is not the only source of secretinase It has long

been known that secretin is ineffective when taken orally (30), it has been found by Doubilet and Ivy (60) to stimulate when given rectally, and is also effective on intramuscular or subcutaneous injection In both instances the latent period is longer and a far higher dosage is necessary for an effect to be manifested Its ineffectiveness on oral administration has always been attributed to the fact that the hypothetical protein molecule was destroyed by peptic and tryptic digestion Greengard, Stein, and Ivy (127), however, observed no destruction of either crude or purified secretin by crystalline pepsin, trypsin or chymotrypsin It is probable that the agent which destroys orally administered secretin is secretinase in the stomach and intestine, which was also present in the pepsin and trypsin preparations used by the earlier investigators, while the much larger quantity required for effectiveness on subcutaneous or intramuscular injection may be on the basis of destruction of most of the hormone before it gains access to the pancreas In this connection it should be mentioned that even when secretin is given intravenously, much of it probably never reaches the pancreas intact The minimal effective dose required to stimulate when injected directly into the arterial supply of the pancreas has never been ascertained

7 *Clinical Applications*

The attainment of a nontoxic secretin preparation free from side actions has long been sought, with a view to employing it as a diagnostic tool for evaluation of the functional state of the pancreas It has been injected intravenously into normal human subjects (5,34,342) with a duodenal tube in place, and in adequate doses under such conditions has abundantly increased the flow of duodenal contents from the tube, and the total output of pancreatic enzymes contained therein A number of investigations (33 36 37,53 54,55 206) have since demonstrated these responses to be diminished in the presence of pancreatic injury or disease, and on this basis it has been found possible to differentiate steatorrhea due to digestive deficiency, as in pancreatic achylia, from that produced by absorptive failure, such as occurs in sprue or celiac disease (17,260) In the presence of obstruction of the pancreatic ducts it is stated to produce an increase in serum lipase, not noted in either an intact or an atrophied gland (275) A test based on the production of a functional obstruction to the ducts produced by the spasmogenic action of morphinization prior to the injection of secretin has been devised by Lagerlof (207), and Friedman and Snape (102) believe measurements of the enzyme production by the gland in response to combined secretin insulin injections to be a more reliable index of pancreatic deficiency than repression of volume output alone

8 *Summary*

Secretin is the hormonal agent stimulating the external secretion of the pancreas, particularly its inorganic constituency Its existence has been established by a series of appropriate experiments, including transplantation studies and isolation in the crystalline state The recrystallized picrolonate has been found to have the empirical formula C_7H_9ON, the structure is as yet undetermined, but it is apparently free of reactive hydrogen atoms Pure secretin stimulates the secretion of the liver as well as of the pancreas The action on the pancreas is not long maintained, apparently due to destruction of the secretin by an enzyme present in the blood and tissues designated as "secretinase" Pure secretin, or highly purified concentrates, have been injected in the human for the purpose of determining the integrity of pancreatic function By this means certain differential diagnoses are feasible

B Pancreozymin

1 *Demonstration*

In the foregoing discussion it has been noted that secretin administration evokes the secretion of an enzyme-poor pancreatic secretion In most early reports crucial data have been lacking, principally because of the inadequacy of methods for the quantitative determination of enzymes, and a few investigators have reported an increased enzyme concentration after secretin (34,206) The proposal of Mellanby that the enzyme output of the pancreas is entirely under vagal control was refuted by Harper and Vass (140), who noted an increase in the enzyme content of pancreatic secretion when food or water entered the duodenum from the stomach, which was unaffected by complete extrinsic denervation of the small intestine Evidence that the enzyme content of pancreatic juice elicited by secretin depended on the method of preparation of the secretin concentrate was obtained by Braga and Campos (22) Friedman and Thomas (103) noted that instillation of peptone in the duodenum elicited the flow of pancreatic juice of higher enzyme content than when dilute acid instillation or secretin injection served as the stimulant of flow

An explanation of the discordant results of previous workers was obtained by the discovery of Harper and Raper (139) that a secretin concentrate prepared by a modification of Mellanby's method could be separated into two factors one of these was secretin, and the other a previously uncharacterized agent which had no effect on the volume output of the pancreas, but operated to increase the amylase concentration of the juice while secretin stimulation was acting, and which they

called "pancreozymin' The hormonal nature of the agent was indicated by the fact that its effect was still manifested after vagotomy or atropinization, and it had earlier been shown by Farrell and Ivy (78) that an autotransplanted pancreas manifests an augmentation of both fluid and enzyme production after a meal Thus enzyme production is established to occur in the absence of all extrinsic innervation of the pancreas Confirmatory evidence of the existence of pancreozymin was obtained (119) by a re-examination of the various fractions separated in the purification of secretin by the process of Greengard and Ivy It was noted that their SI preparation produced a secretion of much higher enzyme content than did purified secretin and that the enzyme stimulating factor was in the fraction precipitated by aniline All three of the chief pancreatic enzymes were elaborated in increased concentration, as was expected

2 *Concentration*

Two methods have been described for the preparation of a pancreozymin concentrate Harper and Raper extracted scraped intestinal mucosa with absolute alcohol, removed the alcohol by vacuum distillation, precipitated the secretin by the addition of bile salts and acetic acid, and saturated the filtrate from this treatment with salt The resulting precipitate was extracted with absolute alcohol, the extract, evaporated to dryness contained the pancreozymin activity Greengard and Ivy (124) noted that the precipitate obtained by treatment of SI in 80% acetone solution with aniline served as a potent source of pancreozymin From this precipitate traces of secretin as well as other contaminants could be removed by extraction with acidified methyl alcohol

3 *Occurrence*

Pancreozymin was found by Harper and Raper to have a source and distribution in the body identical with secretin None was obtainable in extracts of tissues other than the intestinal mucosa

4 *Method of Assay*

Harper and Raper determined the activity of their concentrates by collecting from the cannulated pancreatic duct of anesthetized cats the enzyme-poor secretion elicited by injection at 12 minute intervals of pancreozymin free secretin When the rate of secretion and amylase output became constant, injections of the pancreozymin concentrate were made at intervals, and the magnitude of increase of enzyme output was ascertained Greengard and Ivy (124) devised a method based on the finding that at the height of secretin stimulation the enzyme content of

pancreatic juice is extremely low Dogs were given injections of purified secretin at 10-minute intervals, the secretion obtained in response to the first few of these was discarded, as they served for the washing out of preformed enzymes and subsequent samples were fractionated into 3-minute collections the enzyme contents of which were determined The injections of pure secretin elicited a secretion considerably poorer in enzymes in the second 3-minute period than in the first, but its activity was increased by administering pancreozymin prior to its collection A unit of pancreozymin was defined as that amount which increased the enzyme content of the second 3-minute sample to the level of the first Comparison of the Harper Raper and Greengard-Ivy products by this method revealed them to be of approximately equal potency

5 *Properties*

Concentrates have been found to be water soluble, and insoluble in organic solvents In the dry form the activity has not been shown to deteriorate but it does do so in solution and, like secretin, is much more sensitive to alkali than acid Solutions may be boiled for up to 15 minutes without a decrease in potency Harper and Raper found their product to be resistant to peptic digestion (U S P pepsin) but destroyed by enterokinase activated pancreatic juice Their material was slowly diffusible through a cellophane membrane Harper and Mackay (141), employing biopsy studies, observed that the zymogen granule content of the pancreatic acinar cells was unaffected by secretin injection, and diminished but never exhausted by vagus stimulation or the injection of pancreozymin

6 *Metabolism*

Evidence has been obtained (119) which indicates that pancreozymin, like secretin, undergoes enzymic inactivation in the blood and tissues

7 *Clinical Applications*

The discovery of pancreozymin is at present too recent for the evolution of any complete studies on its applicability Preliminary trials (125) indicate that its administration in the dog increases the concentration of serum enzymes to some extent in the presence of an intact pancreas

8 *Summary*

Pancreozymin has been demonstrated to exist as a hormone entirely distinct from secretin, liberated from the same source and by the same

type of stimulus, and acting on the same end organ Its effect is to stimulate the production of enzymes by the pancreas

C Cholecystokinin

1 *Demonstration*

It was first recorded by Okada (255) that an increased tone of the gall bladder resulted from the ingestion of a meal, or from the application of dilute acid to the intestinal mucosa Subsequently Braga and Campos (22) injected crude secretin preparations, and noted an expulsion of bile from the gall bladder, but there was no indication that this effect did not result from vasodilation, with pressure exerted mechanically on the viscus from the engorged liver Shortly thereafter a number of clinical investigators produced radiological evidence of the evacuation of the human gall bladder promptly after the ingestion of a meal (21,25,38,147) The crucial experiments demonstrating the existence of a humoral mechanism controlling gall bladder evacuation were performed by Ivy and collaborators (151,161,162,163,164), who showed that vasodilatin-free secretin concentrates, when injected intravenously, stimulated the musculature of the gall bladder to contract They correctly attributed this effect to a hormone distinct from secretin, which they called "cholecystokinin," and conclusively proved its existence by appropriate cross circulation and transplantation experiments

The proof of the existence of cholecystokinin is based on the following evidence

(1) An appropriate stimulus applied to the upper intestine, such as dilute acid, fat, or a meal, will cause the gall bladder to contract
(2) These substances, as well as various digestive products, cause no contraction of the gall bladder when injected intravenously (343)
(3) Acid instillation of a denervated loop of intestine will cause a contraction in the autotransplanted gall bladder
(4) Extracts of the intestinal mucosa cause a contraction of the gall bladder when injected intravenously

2 *Occurrence*

Cholecystokinin is obtained from the same source as secretin and pancreozymin no other has ever been demonstrated It is of interest that certain species of animals possess no gall bladder, and that in the case of one of these, the horse, Drewyer and Ivy (61) extracted the intestinal mucosa, tested the purified extract for cholecystokinin activity, and found only traces present From such an observation it might

be generalized that animals lacking a gall bladder produce but little of the hormone which stimulates it It was found present in the human and rabbit intestine (66)

3 *Concentration*

Cholecystokinin was found by Ivy and collaborators (228) to accompany secretin in its purification The SI concentrate contains both secretin and cholecystokinin From such trichloroacetic acid precipitates it was found possible to extract most of the secretin activity with 95% alcohol, leaving an alcohol insoluble residue containing little secretin, at the expense of the inactivation of appreciable amounts of cholecystokinin In the procedure for the isolation of crystalline secretin, Greengard and Ivy (123) and Doubilet (59) noted that the cholecystokinin was present in the butyl alcohol extract of the filtrate from aniline precipitation Ågren (3) prepared a concentrate by a procedure quite similar to the SI method, and reported the activity to be concentrated in the electrodialyzate The hormone has not yet been isolated in the chemically pure form, and it is not yet established that any of the concentrates are biologically pure

4 *Properties*

Cholecystokinin manifests the same characteristics of thermolability, degradation in solution, and sensitivity to alkali as have been noted for secretin and pancreozymin Likewise, it is dialyzable Its characteristic biological effect is the production of a contraction and evacuation of the gall bladder, and this has been followed radiologically in the rabbit by Walsh (344) and in the human by Ivy, Drewyer, and Orndoff (154) As in the case of secretin, the effect is most pronounced by far when it is given by the intravenous route, it is not active by mouth, but has been shown by Doubilet and Ivy (61) to be absorbed and effective when given rectally When added to a bath of oxygenated saline containing suspended strips of gall bladder (Mellanby, 242) or an isolated guinea pig gall bladder (Jung and Greengard, 169) a contraction of the isolated gall bladder tissue was obtained The latter workers found that the response was unattenuated by addition of atropine, which agency completely abolished the response to acetylcholine Sandblom, Voegtlin, and Ivy (292) noted that cholecystokinin caused a relaxation of the sphincter of Oddi, together with an increased duodenal motility as registered by a balloon Whether the sphincter relaxation is due to a direct effect of the hormone or to a reflex from the contracting gall bladder is as yet undetermined, nor is it known whether the effect on the intestine is attributable directly to it

5 *Biological Assay*

The most reliable procedure for the determination of cholecystokinin potency is that devised by Ivy and Oldberg (164), which consists of exposing the gall bladder in its bed, clamping or ligating the cystic duct while excluding the cystic artery, and securing in the dome of the gall bladder a metal trocar about 6–7 mm in width, which is connected to a sensitive recording tambour On such a preparation the increases in tension within the gall bladder produced by cholecystokinin injections

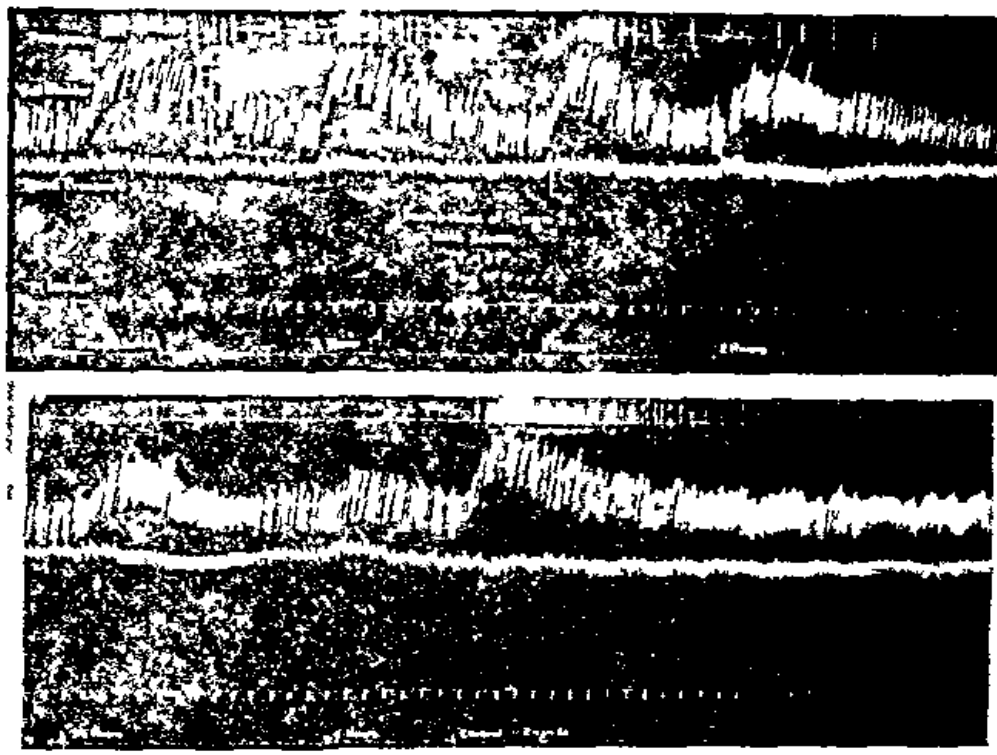


FIG 3.—Progressive inactivation of secretin and cholecystokinin during incubation of SI with dog serum (From Greengard *et al*, 129)

are measured, and the unit is defined as that amount which will produce a rise equivalent to 1 ml of bile in the absence of vasodilation This effect is brought about in most dogs by the administration of 0 3–0 5 mg of SI Assays have also been performed on the isolated gall bladder *in vitro* By such a procedure Ågren (3) noted a contraction on the addition to the bath of 0 4 mg of his best preparation, and Doubilet and Ivy (60) with 0 1 mg of SI

6 *Metabolism*

By the same process whereby the existence of secretinase was established, Greengard, Stein, and Ivy (129) noted that blood serum contains

a principle which inactivates cholecystokinin, and which manifests the behavior typical of an enzyme with regard to pH and temperature effects and thermolability The presence of such an agent may be the factor responsible for allowing relaxation and filling of the gall bladder in the intervals between meals A typical record of the inactivation of secretin and cholecystokinin by serum for various periods of incubation is depicted in Fig 3

7 *Clinical Applications*

As indicated above, intravenously injected cholecystokinin produces a contraction of the human gall bladder which can be followed radiologically (154) Presumably the material would serve as an instrument for nonsurgical drainage of the gall bladder, and as an adjunct to the Graham-Cole test in determining the ability of the viscus to evacuate It is no more potent in so doing than is a Boyden meal of egg yolks and cream, nor is its action appreciably more prompt However when a concentrate of sufficient purity is available in adequate amounts for clinical use, it is not unlikely that a dosage effective in normal individuals can be established to serve as a standard for comparison with the amount required by a diseased gall bladder In this connection, numerous assays on dogs have shown a healthy gall bladder to be much more sensitive to cholecystokinin than an inflamed or fibrotic one

D Enterogastrone

1 *Demonstration*

It was first shown by Ewald and Boas (74) that the addition of olive oil to a meal of starch paste would inhibit gastric secretion and delay gastric evacuation in the human subject Shortly thereafter, Pavlov and his collaborators launched a series of investigations which corroborated and extensively amplified the evidence thus obtained Khizhin (175) noted that meals high in fat elicited a meager gastric secretory response, and that the normal marked secretion elicited by a meat meal was diminished by the addition of fat Lobosov (222) observed a decrease in enzyme content of the juice as well as volume output, and Wirschubski (355) confirmed the inhibition of gastric motility He also showed the inhibition to be followed by a secondary excitation Kasanski (170) found olive oil to inhibit the hypersecretion produced by perfusion of the stomach with warm saline

Up to this time it was assumed that a local effect of fat on the stomach constituted the inhibitory agency This was disproved by Lintvarev (221), who demonstrated inhibition of gastric motility and closure of the

pylorus following introduction of olive oil in the duodenum, which he attributed to a duodenogastric reflex Subsequently Sokolov (311) proved conclusively that inhibition was obtainable only after the duodenum was exposed to the action of the fat, which he did by preparing dogs with a Pavlov pouch and with fistulas of the main stomach and duodenum which he could connect or disconnect at will In such animals there was no inhibition of secretion from the pouch when fat was placed in the main stomach It occurred only when fat was placed in the duodenum, and he believed the inhibitory nervous reflex to originate there His studies were repeated and confirmed by Lonnqvist (226) Orbeli (257) compared the fat inhibition in a Pavlov pouch before and after vagal denervation, and found it to be less in the latter case, which he interpreted as support of the conception of a nervous reflex mechanism, with the vagus nerve constituting the efferent limb of the reflex arc That the duodenum was also the region responsible for the secondary stimulation after the ingestion of fat was shown by Piontkovski (261), who noted excitation following the introduction of soaps therein, and concluded that the biphasic action of a fat meal was due to an initial inhibition occasioned by the presence of neutral fat in the duodenum, followed by a secondary augmentation when the fat was subjected to intestinal digestion and converted to soap

These fundamental observations were abundantly confirmed in all respects, except regarding the mechanism whereby the inhibition occurred, and by a variety of techniques A number of clinical investigators (6,9 35 208 321 333) found the findings applicable to the human stomach, and Cannon (29) obtained radiological evidence of inhibition of peristalsis, both in frequency and degree, after a fat meal was given Carlson (31) and Quigley Zettleman, and Ivy (280) noted that the introduction of fat into the duodenum inhibited hunger contractions as recorded by the balloon technique

The conception that the inhibitory effect of fat was a nervous-reflex phenomenon did not survive The elucidation of its true mechanism originated when Ivy, Lim, and McCarthy (166) obtained inhibition of the secretion of a vagotomized pouch of the entire stomach, and a totally denervated Bickel pouch in response to fat placed in the duodenum Subsequently Farrell and Ivy (77) showed the procedure to inhibit the spontaneous hunger contractions of an autotransplanted gastric pouch, and Feng, Hou and Lim (80) the secretory activity Finally, Quigley, Zettleman, and Ivy (280) demonstrated the persistence in the inhibition of motor activity of a pouch after bilateral vagotomy, bilateral splanchnectomy, and celiac ganglionectomy, as well as in the autotransplanted pouch, and thereby established that fat inhibition could not be accounted

for on a nervous-reflex basis, but must of necessity be due to a humoral agent

Further work revealed the true nature of such an agent The possibility that it represented some digestive product absorbed from the duodenum was ruled out by the experiments of Feng, Hou, and Lim (80), who showed that no inhibition occurred when thoracic duct lymph was injected intravenously, and that a thoracic duct fistula did not alter the effects of fat administration hence absorbed fat passing through the lymph vessels was not the inhibitory agency The presence of bile discharged into the duodenum as a result of fat administration was shown to be unrelated to the inhibition It was shown by Sokolov (311), by Meyer, Ivy, and McEnery (245), and by Kosaka and Lim (192) that bile in the intestine stimulates, rather than depresses gastric secretion Other nonspecific agents were ruled out by Quigley, Zettleman, and Ivy (280), who noted no inhibitory effect in response to injections of fat, lymph, soap, or glycerol Secretin and cholecystokinin in ordinary doses were ineffective

Thus all known absorptive products were excluded as possibly being the humoral agent involved, and the existence of a specific chalone was strongly suggested In support of such a conception, Kosaka and Lim (193) injected large doses of Ivy's cholecystokinin and found it to inhibit the secretion from a Heidenhain pouch This led to further studies (194,195) in which they prepared saline extracts of duodenal mucosa previously exposed to olive oil and found them to be active, whereas extracts not thus exposed, or extracts of gastric mucosa, were ineffective These findings clearly indicated the existence of a specific principle elaborated into the blood stream in response to exposure of the duodenal mucosa to fat, which they named "enterogastrone" The presence of enterogastrone in the blood has been indicated as a result of experiments by Tschukitscheff (334), who obtained inhibition of hunger motility in a dog after a transfusion of blood from a fed animal, and by Kosaka and Lim (194), who precipitated active material from the blood of a fat-fed dog

2 *Occurrence*

The site of enterogastrone formation is almost entirely in the duodenum, on the basis of several experiments Ivy, Lim, and McCarthy (167) found the inhibitory effect on a Pavlov pouch of coating the stomach with lard to be slight, and Farrell (76) made a similar observation on a total pouch Smidt (310) resected the pyloric antrum in Pavlov pouch dogs and noted the inhibitory effect of fat to persist, whereas if the first part of the duodenum were also resected it disappeared Kosaka, Lim,

Ling, and Liu (195) extracted a number of tissues, and found the only extracts effective were those of the intestinal mucosa, thus establishing it as the site of enterogastrone elaboration

3 *Concentration*

The original preparation used by Kosaka and Lim for an active extract was the SI concentrate previously referred to Kosaka, Lim, Ling, and Liu (195) and Lim, Ling, and Liu (216) reported the preparation of a concentrate which was potent and practically free of vasodilatin, secretin, and cholecystokinin by picric acid precipitation from a saline extract of mucosa from a fat-instilled duodenum, decomposition of the picrate with acidified alcohol, and acetone precipitation Gray, Bradley, and Ivy (111) obtained a superior product in a greatly improved yield from the same "A-precipitate" employed as a source material for the other hormones, by a procedure of suspension in water, isoelectric precipitation, heat coagulation, separation of the activity from the filtrate as an insoluble tannate, and decomposition of the latter with acidified acetone The product was potent in repressing gastric secretion and motility in experimental animals when injected intravenously, it was also effective on subcutaneous or intramuscular injection, but in the dosage necessary to produce such an effect was markedly irritating Further concentration of the activity was effected by Greengard and others (121) employing precipitation with picric acid of the product of the above procedure The precipitate on treatment with acidified acetone was separated into an active extract and an insoluble residue in which most of the irritant material remained From the extract the activity could be precipitated by an excess of acetone in the form of a freely soluble colorless powder, approximately twice as potent as the original product in depressing gastric secretion

4 *Biological Assay*

Methods have been devised for the evaluation of activity of enterogastrone concentrates based on their potency in repressing the motor and secretory activity of the stomach Kosaka, Lim, Ling, and Liu (195) employed as a criterion of activity the duration of inhibition of hunger motility in dogs, and the extent of inhibition of secretion from a Heidenhain pouch after a meal Gray, Bradley, and Ivy (111) obtained more consistently reliable information by determining the motor inhibitory effect on gastric peristalsis induced by placing a balloon in the stomach and inflating it with 80–100 ml of air, and on secretion by the use of dogs with pouches of the entire stomach The method first devised for secretory inhibition consisted of injecting the dogs at 10-minute intervals

with a dosage of histamine adequate to elicit a gastric secretory response of 1 ml per minute When this was established as a steady rate, the enterogastrone concentrate was injected and the degree of inhibition noted The unit was defined as that quantity which reduced the output of free HCl by 50% for 2 hours following injection This procedure has more recently been modified to one in which the pouch dogs are given two single injections of histamine spaced about 5 hours apart It was shown by Wells Gray and Dragstedt (346) that on any given day the responses to the two doses are essentially alike, although any dog will fluctuate from day to day Prior to the second injection the entero gastrone concentrate is injected, and the response to the second histamine injection is compared to the control value The unit of enterogastrone derived by this method is defined as the amount which will halve the response to the second injection of histamine with reference to a control response of 40–120 mg of HCl in 90 minutes and is essentially identical with the unit as measured by the continuous-injection technique Fried man and Sandweiss (100) have devised an assay method based on determining the effectiveness of the material in inhibiting the spontaneous gastric hypersecretion induced in the anesthetized rat by pyloric obstruction

5 *Properties*

The most potent concentrate obtained is a colorless powder freely soluble in water to give a colorless solution It has been found to be diffusible through a cellophane membrane (120) indicating that the molecule is probably not very complex It is insoluble in strong methyl or ethyl alcohol and other organic solvents Apparently the active principle is more stable in solution than secretin or cholecystokinin, for aqueous solutions have been stored for up to 10 days in the sterile state at a slightly acid reaction with no detectable diminution in activity In alkaline solution it undergoes rapid degradation In acid solution it is resistant to boiling of duration up to 30 minutes It is absorbed by a variety of insoluble solids, and destroyed by peptic digestion

The physiological properties of enterogastrone concentrates will be considered under three topics, as indicated below

(1) *Effect on gastric motility* Both hunger motility and digestive peristalsis are inhibited by intravenous injection of the tannic acid purified product The duration of inhibition was found by Gray, Bradley, and Ivy (111) to be dependent on the dosage The two types of motility were found to be equally affected It was originally believed that a single agent was effective in the depression of both motor and secretory activity, however the product obtained through picric acid

purification manifests an enhanced potency in inhibiting secretion and a greatly diminished one on motility Furthermore, the SI concentrate is a potent motor inhibitor in small doses, which may be many times multiplied without manifesting any inhibitory effect on secretion induced by a meal or by histamine (120) These circumstances clearly indicate the existence of two separate principles

The motor inhibitory effect of enterogastrone concentrates has been demonstrated to depend on the integrity of the vagus innervation to the stomach The intact stomach and a vagally innervated pouch are inhibited, either by the injection of active extracts or by instillation of fat into the duodenum, whereas the motility of a denervated pouch is unaffected by the injection of extracts (142) but still is inhibited by fat in the duodenum Motor inhibition may be obtained from either intravenous or subcutaneous injection The latter manifests a longer latent period and slower recovery, and the effective dosage is about four times as large

(2) *Effect on gastric secretion* The inhibitory action of enterogastrone on gastric secretion is manifested on both innervated and denervated pouches, and is effective regardless of the nature of the stimulus applied when the dosage is adequate The extent and duration of the effect depend roughly on the dosage employed, however, no satisfactory concentration action ratio can be constructed, owing to the inherent variability of the process of gastric secretion in different animals, and in the same animal from day to day

The effect of enterogastrone on the composition of gastric juice was investigated by Gray, Bradley, and Ivy (111), who determined the volume output and the contributions of parietal and nonparietal secretion thereto, and concluded on the basis of their determinations that enterogastrone exerted a selective inhibitory effect on the parietal secretion, since their calculations revealed that the decrease in volume and free acid was consistent with such a situation Whether the enterogastrone entered the circulation of the dogs as a result of injection of a concentrate or of invoking the animal s intrinsic enterogastrone mechanism by fat instillation there was always a greater diminution in the output of free acid than in the volume output Thus the concentration of acid in the postenterogastrone collections was reduced, and by dividing the secretion into parietal and nonparietal components on the basis of a concentration of HCl of 6 mg per ml in pure parietal secretion, it was noted that the output of nonparietal secretion was essentially unaltered after enterogastrone Furthermore, the output of mucus, a typical nonparietal constituent, remained constant Subsequently, it was noted (133) that the output of pepsin was only slightly diminished by entero-

gastrone in animals with vagally denervated pouches, but markedly in the case of pouches with the vagus innervation intact In the light of these findings, it appears that pepsin inhibition, like inhibition of motility, is dependent on the integrity of the vagi However, a denervated pouch invariably has a lower pepsin output than an innervated one

(3) *Effect on the resistance of the alimentary tract to ulcerative lesions* In view of the therapeutic potentialities of enterogastrone as a means of attenuating the gastric hypersecretion accompanying peptic ulcer, an investigation was made regarding its efficacy in preventing the development of gastrojejunal ulcer in dogs prepared by the Mann-Williamson operation, a procedure which uniformly results in ulcers within a period of about four months It was found (137) that, when two daily injections of the tannic acid product were administered, 80% of the animals remained in good condition and did not develop an ulcer during a year's observation, whereas, in a control group of animals given the same dose of hog muscle extract prepared by the identical procedure, an ulcer developed in all cases, showing that the protection was not of a nonspecific nature The fact that protection failed in 20% of the animals was considered to be due to their becoming refractory to enterogastrone, a condition which Gray and Wieczorowski (113) had shown to obtain in this proportion of pouch dogs employed for assay of the material The injections were discontinued after a year, in order to permit the animals to develop ulcers and then to ascertain if these could be cleared up by resumption of the treatment, as had been done in previous investigations on other forms of antacid therapy At this time the animals were explored and found to be free of ulcers It transpired that the protection afforded by the course of enterogastrone injections was a lasting one Most of the dogs died of causes other than ulcer many months after cessation of treatment A few developed ulcer from 1½ to 3 years later, thus indicating that no real adaptation of the animals to the operation had taken place

These results indicated that the protection afforded to Mann Williamson dogs is not attributable to an antacid effect of enterogastrone alone, since the action of enterogastrone in the dosage used was of only a few hours duration, and since other forms of antacid therapy continued for the same interval fail to give lasting protection Thus the presence of a hitherto uncharacterized principle which increases the resistance of the mucous membrane to injury and promotes healing was strongly indicated In a subsequent series of experiments (151) the animals were observed to be protected by a single daily intramuscular injection of the above concentrate after further purification through picric acid In this series protection was obtained in all animals The mechanism of the protective

influence has been investigated, and the only significant finding was the fact that whereas an untreated Mann-Williamson dog manifests a continuous hypersecretion in response to an alcohol test meal (309,353) such a dog after a course of treatment with enterogastrone evidences a response similar to that of a normal dog (132) (Fig 4) This indicates a modification of the activity of the parietal cells, but the means whereby this is brought about remains obscure Recent studies (unpublished) have indicated enterogastrone treatment to stimulate mucus formation at the site of ulcers produced in rabbits by excision of a piece of gastric mucosa

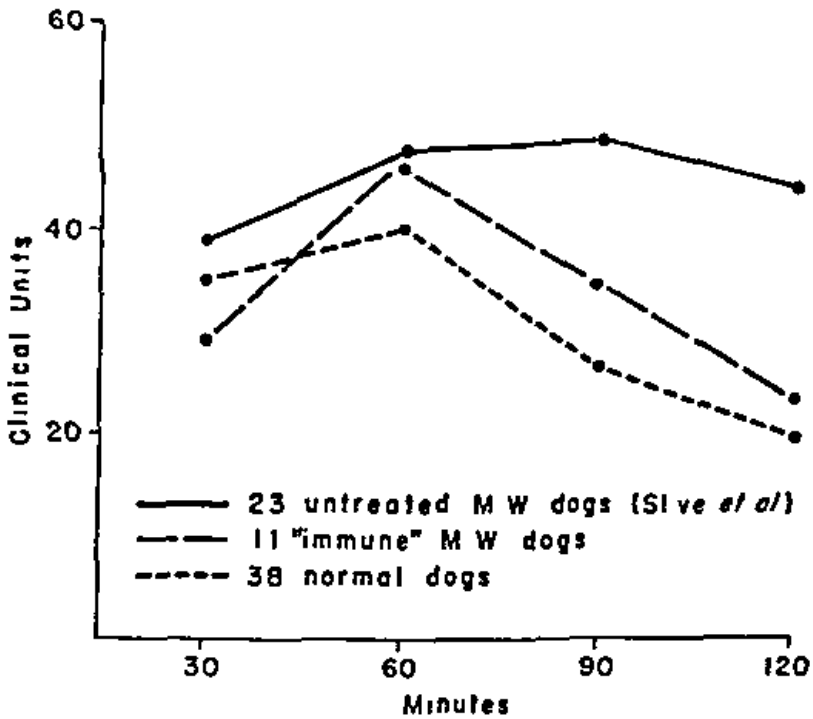


Fig 4 —Output of free HCl in treated and untreated Mann Williamson dogs, compared with that of normal dogs in reoponse to an alcohol test meal (Redrawn from Grossman et al , 132)

Following the observation of protection against experimentally produced ulcers in dogs, the same procedure was applied to patients with proved peptic ulcers of long standing This investigation (117) revealed that although enterogastrone injections are no more effective than other forms of antacid therapy in relieving the acute symptoms of peptic ulcer, there is apparently a tendency to lasting protection when injections are continued over a period of a number of months

In summing up the properties of enterogastrone concentrates, apparently the less highly purified concentrates contain three agencies, including motor inhibitory and secretion inhibitory factors and in addition an

agent with the protective effect on the mucous membranes indicated above

In fact this latter agent is undoubtedly separate and distinct from the substances which inhibit gastric motility or secretion The daily dosage employed in the treatment of ulcer in the dog or the human would be capable of inhibiting the stomach for only an hour or two and to an extent far from complete especially in a hypersecreting patient or animal Furthermore the protection against recurrent attacks which prevails beyond the period of daily injections cannot possibly be attributed to inhibition of gastric motility or secretion Thus the evidence strongly indicates the existence of another factor either in the form of another action of enterogastrone or of another substance present in the concentrate employed Support to this view is given by the apparent protective effect of crude extracts of the intestinal mucosa when administered orally to the Mann Williamson dog (117) the inhibitory action of enterogastrone on the stomach cannot be obtained when it is given orally and it has been shown to be susceptible to peptic digestion (142)

In view of the evidence favoring the existence of a separate antiulcer factor it is apparent that the assay methods cited previously are capable of providing only an indirect measure of potency in this regard Prior to 1945 the only direct procedure involved the preparation and prolonged daily treatment of Mann Williamson dogs with a lapse of several months before any indication of effectiveness was obtainable At that time Shay and associates (308) noted that acute gastric ulcers were producible in rats by pyloric ligation after a preliminary period of starvation lesions occurred uniformly in the rumen inconstantly in the antrum and least often in the fundus They were attributed to the continual presence of unneutralized gastric juice since they could be also produced by instillation with rat or human gastric juice or acid pepsin solution The protective effect against such ulcerations produced by pretreatment with active extracts has been studied by several investigators (285 340 350) in general the degree of protection afforded by enterogastrone concentrates against such lesions has been apparent only when large amounts are given An assay method based on the production of a single acute ulcer in the rat by the injection of dilute phenol into the stomach wall has been devised by Frederick and Greengard (94) They found that active extracts accelerated healing of such a lesion when injected for a week prior to and following the phenol treatment

6 *Clinical Applications*

From the preceding discussion it is manifest that the therapeutic possibilities of enterogastrone in the management of peptic ulcer are

great The high incidence of ulcer in the general population, the chronicity of the condition, and the inability of all present forms of management to prevent recurrences are well known, and, if sufficiently extensive studies confirm the prolonged protection afforded by enterogastrone, its position in ulcer management will be secure Recently it has been reported (97,108) that the oral administration of intestinal extracts was found of benefit in ulcerative colitis, indicating that the production of increased mucosal resistance or promotion of healing by its use may prevail throughout the alimentary tract

E Action of Intestinal Extracts on Intestinal Secretion and Motility

It has long been known that the principal factors concerned in regulation of the secretion of intestinal juice are mechanical and nonspecific chemical stimuli which act locally (150) However, there is abundant experimental evidence that humoral agents are to some extent operative, and that one or more hormones are concerned in the elaboration of Brunner's gland fluid as well as of succus entericus It was noted many years ago by Ponomarew (263) that feeding would slightly increase the secretion from an isolated segment of duodenum, and these observations were verified and extended by Florey and Harding (90) in the dog cat, pig, and goat It was noted by them also that the response persisted after denervation (91) and after transplantation (92) This evidence establishes the existence of a humoral factor in the case of Brunner's glands, but this is not necessarily a hormone Some support for the existence of a hormone exists, since it has been demonstrated that some secretin concentrates stimulate Brunner's glands when injected intravenously (93,356) The SI preparation is effective in this regard, whereas crystalline secretin is not (313)

The existence of a humoral mechanism stimulating the secretion of succus entericus was definitely demonstrated by Nasset, Pierce, and Murlin (252) who prepared animals with subcutaneous transplants of jejunal and ileal loops, and noted the effect on these of feeding No response was noted prior to complete denervation, but when this was produced by the procedure of severing the original pedicle there was a definite increase (60% over the basal rate) after feeding, and an increase in enzyme output which exceeded the volume increase (305) Subsequently Nasset (251) prepared an extract of intestinal mucosa which stimulated the secretory activity of the intestinal glands, the effect of which persisted after denervation and transplantation, and to which he gave the name "enterocrinin" Processes of purification which have since been applied (83,85, have evolved a highly potent concentrate which

is reported to be free of secretin and vasodilatins The most highly active preparation was that obtained by Fink (83), who employed a procedure of acid alcohol extraction of the intestinal mucosa, precipitation by salt saturation, acetic acid extraction of the dried salt cake, and ether precipitation of the extract The product was subjected to subsequent extractions with acetic acid, methylcellosolve, and propylene glycol, followed by picric acid precipitation and decomposition of the isolated picrate with acetone, hot water, and acetic acid Certain batches obtained according to this procedure were potent in a dosage of 0 036 mg

The only method described for the biological assay of enterocrinin is that of Fink and Nasset (84) involving the insertion of a wire coil into a jejunal loop to maintain patency of the lumen, which was then filled with saline and subjected to gentle suction When it reached a steady state, the material to be tested was injected and any increase noted Any method for assay is handicapped by the fact that a mechanical stimulus of the intestinal mucosa is incomparably more potent in exciting the flow of juice

A further complicating factor in studying intestinal secretion induced by injected substances is the fact that many extracts of intestinal mucosa will produce an increased motor activity of the intestine (90), and it is in many cases equivocal whether an augmentation of secretion represents a true increase, or a spurious one resulting from the expression of preformed material The augmented motility is apparently in the nature of rhythmic segmentation, since a decrease of passage time of a bolus through a Thiry-Vella loop has been observed not to take place (341) Recently a partial dissociation of the motor and secretory effects has been observed, the SI preparation stimulates both motility and secretion in a freshly prepared solution, but if such a solution is permitted to stand for some time at room temperature, or if it is boiled for half an hour, the secretory component is lost while the motor one persists (122)

It has been reported (72,73,231,232,330,359) that some secretin preparations when injected intravenously will stimulate intestinal peristalsis, and this has been attributed to a hormone designated "peristaltin" or "enterocin" The existence of such an agent is most unlikely *It is a well-known fact that augmented intestinal motility occurs after* feeding but only in a loop of intestine remaining in continuity with the rest of the alimentary tract, it is not seen in an isolated loop, whether innervated, denervated, or transplanted (152) The most potent exciting agents of intestinal peristalsis are locally acting stimuli of a chemical or mechanical nature, whose action may be abolished by local anesthetization (19)

Another aspect of motor activity of the small intestine has been

investigated by Kokas and Ludany (183,184,185,186,187), who postulated the existence of a humoral mechanism stimulating movements of the villi They noted by observation under a binocular microscope that activation occurs only in segments of the intestine exposed to acid chyme, and that it could likewise be produced in an isolated loop of jejunum joined to the circulation by the carotid artery and jugular vein, following the instillation of dilute acid into the duodenum Cross circulation experiments yielded findings indicating that acid placed in the intestine of one animal activated the villi in both, and the injection of crude secretin concentrates was found to stimulate movements of the villi The active principle was stated to be distinct from histamine, choline, secretin, cholecystokinin, and adenylic acid, and was considered to be a previously uncharacterized hormone designated "villikinin" They considered the mechanism important in the light of experiments showing increased intestinal absorption as a result of augmentation of activity of the villi No confirmatory evidence for the existence of villikinin has been published, and in partial refutation of the studies described above, Loew, Gray, and Ivy (224) noted no acceleration of glucose absorption following instillation of dilute HCl into the duodenum

F Action of Intestinal Extracts on Splenic Contraction

A few investigators observed that the injection of crude secretin concentrate operated in the direction of altering the cellular composition of the blood, as evidenced by a transitory though definite increase in the red cell count (63,64,105,179) It was suggested by Krzywanek (197) that this was occasioned by entry into the general circulation of the erythrocyte-rich blood in the splenic reservoir, as a result of contraction of the spleen by some substance present in the concentrate, since the increase in erythrocyte count was only 4% in the splenectomized dog as compared with 30% in the intact animal In support of this conception, it was noted by Ferguson, Ivy, and Greengard (82) that a definite contraction of the spleen registered either by a splenic oncometer on the organ *in situ* or by isolated strips of spleen in the bath, was obtainable following administration of certain secretin concentrates The SI concentrate was effective in this regard, whereas highly purified secretin was not The action of SI could not be inhibited by atropine

This finding, together with the actions of intestinal extracts on the intestinal musculature and that of the gall bladder, constitute strong presumptive evidence that a single principle is the effective agent, rather than a multiplicity of hormones The proof of such a conception must await the isolation of cholecystokinin in chemically pure form

G Action of Intestinal Extracts on Blood Sugar

A number of workers have obtained evidence purporting to show the existence of a hypoglycemic substance in extracts of the intestinal mucosa, and inferentially a control by the duodenum over carbohydrate metabolism through the medium of a hormonal agent According to some reports (45,46,306) extracts effective when given orally as well as parenterally have been secured, the active principle of which has been termed "duocrine" Others have obtained extracts effective only on parenteral injection (144,145,198,199,200,201,202,203,204,211,212,213,247,254,300, 301,322,323,325,326,327,360,361) termed variously as "incretin," "duodenin," "insulotropic hormone," or "hypoglycemic secretin," and in some instances the effect has been attributed to contamination of the extracts with insulin (159)

Previous discrepancies existing in the literature (18 199,205,307) regarding the possibility of release of a substance by an acid instilled duodenum which counteracts induced hyperglycemia have been resolved by a series of well controlled experiments by Loew, Gray, and Ivy They found, after minimizing all extraneous factors which might modify the blood sugar level, that instillation of dilute HCl into the small intestine was without effect on hyperglycemia induced by alimentary absorption of glucose, by intravenous injection of glucose, by adrenalin injection, or by pancreatectomy (224) The procedure of acid instillation was likewise without effect in lowering the fasting blood sugar level (223) These findings provided a crucial basis for the exclusion of any hormonal influence exerted by the duodenum on carbohydrate metabolism, but not necessarily for the presence of a hypoglycemic substance in duodenal extracts However, it was subsequently shown by these workers (225) that concentrates prepared by many of the procedures alleged to have yielded active material were ineffective in depressing the blood sugar level The only hypoglycemia producing extracts obtained were those of pancreatic tissue Thus, only one possibility remains for any conceivable role which the duodenum might play in exerting any influence over carbohydrate metabolism, which is that it might produce an insulin synergist This question has not yet been investigated

III. The Gastric Mucosa

The existence of two principles of hormonal nature has been postulated for the mucosa of the stomach The presence of one of these, secretin, has been satisfactorily demonstrated by experiments outlined above It has been termed by some "gastric secretin," but there is no

reason to believe that it is not one with the secretin isolated from the small intestine

The Gastrin Theory

Shortly after the secretin mechanism had been demonstrated by Bayliss and Starling, the gastrin theory was advanced by Edkins (69) on the basis of experiments in which he found that extracts of gastric mucosa were effective in stimulating gastric secretion in the anesthetized cat The pyloric mucosa was found to be the most effective, whereas extracts from the cardiac and fundic portions were less active or ineffective Extracts from all three regions of the stomach had a marked vasodilator effect, but those of the fundic region manifested this property the most markedly, often killing the animal Subsequently Edkins and Tweedy (70) separated the fundic from the pyloric portion of the stomach by a flat balloon and cannulated the two portions separately The fundic portion was filled with saline, the pyloric portion with a number of different substances, and the fluid in the fundic portion withdrawn after each test and examined for the presence of free acid It was found to be secreted when meat extract, peptone, dextrin, glucose, or dilute HCl was placed in the pyloric pouch, but there was no local effect following their introduction in the fundic pouch Edkins believed these experiments to demonstrate the existence of the hormone gastrin, elaborated by the pyloric mucosa

A comparison of these studies with those of Bayliss and Starling reveal the evidence obtained by Edkins to be meager in comparison However, the problem has since been extensively investigated and a considerable amount of new material favoring the gastrin theory has accumulated Analysis of the present status of the problem involves a consideration of the following questions

(1) Is the pylorus especially susceptible to stimuli affecting the secretion of the fundic glands?
(2) Is there a humoral mechanism for the stimulation of gastric secretion?
(3) What is the site of action for stimuli which elicit the humoral mechanism?
(4) Does a hormone mechanism for gastric secretion exist?
(5) Is histamine involved in the normal gastric secretory process?

These questions will be considered in order

(1) *Is the pylorus especially susceptible?* A number of experiments have demonstrated conclusively that when secretagogues are applied to the pylorus the fundic glands are stimulated Savitsch and Zelony

(303) caused secretion in a gastrostomy when substances of this type were placed in an innervated pyloric pouch, and Lim, Ivy, and McCarthy (215) obtained a similar response when a denervated pyloric pouch was used Steinberg and collaborators (315) made the same observation, using animals with a fundic and pyloric fistula Priestley and Mann (277) compared the secretory behavior of dogs with a transplanted fundic pouch and with a fundic fistula in response to the introduction of secretagogues into a denervated pyloric pouch They found the transplant failed to secrete, and the secretion obtained from the fundic fistula was meager and sporadic They attributed the secretory response obtained to psychic influences However, their transplanted pouch was decidedly refractory, responding very poorly to histamine

The fundus of the stomach has likewise been shown to respond to the application of secretagogues to it, but considerably less than when these are placed in the pylorus A number of investigators have attempted to compare the behavior of the fundic glands before and after extirpation of the pyloric antrum (176,276,310 354) The balance of the evidence has demonstrated a reduction of a magnitude so minor as to be attributable to the effects of the operative procedure Furthermore, it has been shown that the presence of secretagogues in the intestine will stimulate the fundic glands This was demonstrated in the case of the Pavlov pouch by Ivy and McIlvain (168) and in the total pouch by Ivy, Lim, and McCarthy (167) and by Kim and Ivy (178) However in the case of the duodenum the latent period is long and the response is not great, which may be due to nervous or hormonal inhibitory influences

The experiments above cited indicate a sensitivity to chemical stimuli in the pylorus, fundus, and duodenum, but that of the pylorus very definitely transcends the other two

(2) *Is there a humoral mechanism for gastric secretion?* The fact that the fundic glands could secrete independently of extrinsic innervation was first demonstrated by Popielski (274), who found secretion to occur after bilateral vagotomy, sympathectomy, celiac ganglionectomy, and transection of the spinal cord at the level of T11 He concluded on the basis of his findings that the intrinsic plexuses were involved Similar observations were made by Friedenthal (*95,96*)

Attempts to demonstrate by transfusion experiments the presence of a blood borne stimulus have failed to supply any additional evidence Lim (214) obtained no stimulation on transfusing the blood from a fed donor cat to a fasted anesthetized recipient, and Ivy, Lim, and McCarthy (167) found that transfusion from a fed dog to a Pavlov pouch dog and cross-circulation experiments both failed to give stimulation Razenkov (282,283) reported stimulation in one of three experiments in which he

transfused defibrinated arterial blood from a fed donor to a Heidenhain pouch dog, while a control transfusion from a fasted dog gave negative results Lim and Necheles (219) tested a vividialyzate from the blood of fed dogs on the secretion of a Heidenhain pouch and reported occasional stimulation Their work has never been confirmed

The first conclusive demonstration that a humoral mechanism actually exists was made by Farrell and Ivy (79) when they noted that a subcutaneously transplanted gastric pouch would secrete in response to a meal This observation was confirmed by Lim, Loo, and Liu (217) with homo- and autotransplanted pouches prepared by vascular anastomoses, and by Klein and Arnheim (181) with a transplanted pouch consisting of the mucosa alone Finally, Gregory and Ivy (131) prepared dogs with a transplanted fundic pouch and a Heidenhain pouch of the remainder of the stomach The transplanted pouch was caused to secrete in response to a meal and to application of secretagogues to the Heidenhain pouch, indicating again the existence of a humoral agent When the pyloric portion was separated from the main pouch and constructed into a pyloric pouch, a secretion of the transplant was still observed following perfusion of the main pouch with secretagogues, indicating that the pyloric antrum was not indispensable

(3) *Where is the site of action of the humoral stimulus?* It is definitely known that humoral stimuli are not involved in the cephalic phase of gastric secretion, since this is completely abolished by vagotomy, after which procedure sham feeding evokes no response Regarding the gastric phase the best evidence indicates that the local action of secretagogues is not abolished by denervation The application of a local anesthetic was found by Savitsch (302) to abolish the effect Mechanical distension of the stomach has also been demonstrated to stimulate secretion locally in the absence of the extrinsic nerves Ivy and Farrell (155) and Lim, Loo and Liu (217) obtained this effect in a transplanted pouch, and Gregory and Ivy (131) observed that it was abolished in a Heidenhain pouch by procainization In the case of the intestinal phase of gastric secretion it has been observed by Gregory and Ivy (131) that the ingestion of a meal or the oral administration of secretagogues evoked a secretory response in their dogs both in the transplanted pouch and in the main pouch after procainization

It is thus apparent that the humoral stimulus is operative independent of the innervation of the stomach

(4) *Does a hormone mechanism for gastric secretion exist?* It remains to be determined whether the established humoral mechanism is on the basis of the absorption of nonspecific secretagogues or of the elaboration of a specific hormone or hormonelike substance and whether, if the

latter, it is one with histamine The importance of this last considera tion will be manifest in the discussion which follows

It has been established by numerous investigators that many of the most commonly employed secretagogues, such as meat extract, liver extract, peptone, etc , are effective stimulators of the gastric glands when injected parenterally However, the activity of most of these is attributable to their histamine content, which is adequate to stimulate when given by the parenteral, but not the oral, route Kim and Ivy (178) found the injection of a histaminefree liver extract was very effective They also concluded from their observations that secretagogues might act by absorption from the intestine, but not from the stomach, since they do not disappear during perfusion into a pouch Butler, Hands and Ivy (26) found that liver extract perfused into a pouch was four times as potent in stimulating the gastric glands as when it was injected intravenously, which strongly indicated that an agency other than simple absorption was concerned The concept of a hormonal agent is further supported by the findings of Gregory and Ivy (131) in connection with procainization of the pouches in their dogs They found the secretagogue responses in both the main pouch and the transplant were abolished when the main pouch was procainized prior to its perfusion However, the application of procaine to the transplant, or the subcutaneous injection of procaine, did not prevent the transplant from secreting in response to the presence of secretagogues in the main pouch *It is unlikely that* procaine acts by preventing the absorption of secretagogues, since it does not interfere with the absorption of such substances as ethyl alcohol or histamine, and likewise, it cannot prevent secretion by poisoning the parietal cells, otherwise it would be effective regardless of how it was administered In other words, the evidence cited above indicates that the humoral agent is not identical with absorbed secretagogues, therefore it must of necessity be a hormone

Attempts have been made to verify the existence of a hormone by stimulation with a nonabsorbable agent It was found by Lim, Chang, Hou, and Feng (218) that the feeding of broken bones in conjunction with a meal resulted in an augmentation of the basal secretion, some 18 hours later This was shown to be a mechanical effect, since powdered or incinerated bones were without effect, and inert objects, such as pigs' hoofs, hard rubber, and brass tubing were effective, and the response was due to a humoral agent, since it could be obtained with a transplanted pouch Since the bones could not be absorbed out acted nevertheless by a humoral mechanism, it was concluded that a hormone was involved, and further evidence was provided by the finding that removal of the pyloric antrum abolished the response to bone feeding However, the pro-

cedure was decidedly unphysiological, and the interpretation of the results is questionable, especially in view of the long latent period and the probability that delayed gastric evacuation or obstruction may have permitted the prolonged absorption of secretagogues, since the bones were fed with a meal

The other line of investigation designed to prove the existence of a gastric hormone has taken the form of numerous attempts to isolate the active principle from the gastric mucosa The first such extract was made by Edkins (69) by a procedure very similar to that of Bayliss and Starling, namely maceration with hot 0 4% HCl and neutralization The extract was highly toxic and contained considerable vasodilatin, including the most powerful known stimulant of gastric secretion, histamine All investigators have confirmed the effectiveness of injection of such an extract, the action of which is atropine resistant Any belief that pyloric extracts were specific was soon dispelled by the studies of Popielski (273), Rogers and collaborators (286,287,238), Tomaszewski (331,332), Keeton and Koch (172), and Luckhardt, Keeton, and Koch (227), who demonstrated that potent extracts could be prepared from almost any organ or tissue of the body by the same method In addition to their effect in stimulating gastric secretion, a lowering of the blood pressure and prolongation of coagulation time of the blood was manifested by all such extracts, including Edkins' Dale and Laidlaw (43) compared the properties of histamine with the vasodilatin obtained from tissue extracts, and concluded that the two were identical Popielski (274) agreed with their conception, and believed that histamine was the active principle in all extracts stimulating gastric secretion However, Maydell (235) claimed to have verified Edkins' work in that he found only pyloric extracts effective in stimulating a chronic gastrostomy preparation, and Lim (199) stated that pyloric extracts were more active than cardiac, fundic or duodenal extracts Keeton, Luckhardt, and Koch (173), on the basis of meager chemical evidence, believed that gastrin as represented in their pyloric extracts was not histamine They noted some slight differences in regard to their precipitation behavior by *picric and picrolonic acids*

These studies were climaxed by the isolation of a crystalline compound by Sacks, Ivy, Burgess, and Vandolah (290) the picrate of which was found to be identical with histamine picrate by a mixed melting point determination They observed throughout their purification process that the vasodilatin and gastrin potencies, as well as the Pauly reaction for imidazoles, ran closely parallel, and found the activity of their extracts to be abolished by incubation with histaminase The parallelism between gastrin and histamine assays and susceptibility to histamin

ase was confirmed by Gavin, McHenry, and Wilson (106), who found much more histamine in the fundus than in the pylorus Histamine was first isolated from gastric mucosa by Abel and Kuboda (1) but the contribution cited above represents the first successful effort to isolate it from the pyloric mucosa The evidence obtained by the parallelism between vasodilator and secretory effects, and the complete destruction of activity by histaminase, is strongly presumptive evidence that histamine was the only gastric excitant present in the extracts It is not conclusive, since histamine is not a specific substrate for histaminase, which is in fact a diamine oxidase, and it may have destroyed gastrin as well as histamine

Recently the preparation of histaminefree gastrin preparations has been reported Komarov (188–191) employed a procedure which he considered gave a concentrate of the protein fraction of pyloric extracts, which he effected by extraction of the minced mucosa with dilute acid, addition of base beyond neutralization precipitation of the activity with salt and acetic acid, extraction of the precipitate with 80% acetone, and reprecipitation of the active principle from the acetone freed extract with trichloroacetic acid Solution of the resulting material in methyl alcohol, followed by ether precipitation, was stated to give a product free of histamine and otherwise biologically pure, which was potent in stimulating gastric secretion in 200 mg doses and was atropine resistant Extracts from the fundus of the stomach, and extracts of liver, were found ineffective An examination of Komarov's protocols reveals that the gastric secretory responses were of a very low order, and other investigators have been unable to obtain active extracts by this method Recently, however, Uvnäs and co-workers (10,250,335–337) have prepared active extracts of pyloric mucosa by dilute acid extraction, salt precipitation, solution in water, and reprecipitation by tannic or trichloroacetic acids, 80% alcohol extraction of the resulting material, and isoelectric precipitation at a pH of 8 The product was described as histaminefree, proteinlike, heat stable, and destroyed by pepsin, trypsin, ultraviolet light, and alkali It was obtainable from the pyloric mucosa of cats, dogs, and pigs and was stated to stimulate selectively the parietal cells It is conceivable that Komarov and Uvnas have isolated a histamine liberating protein split product, this would account for its atropine-resistant behavior Friedman and King (98) also reported the preparation from the pyloric mucosa of a gastrin concentrate, effective in a dosage of 60 mg , in this quantity of material there was less than 0 04 micrograms of histamine according to an assay based on its effect on the blood pressure of the cat

To summarize the evidence cited above, it appears that the activity

of Edkins' original extract was undoubtedly due to histamine, and that extracts made by such a procedure contain no other gastric stimulant Acceptance of the existence of an agent other than histamine must await full confirmation of the findings of a histaminefree agent, and its concentration to an order of potency commensurate with that of histamine

(5) *Is histamine involved in the normal gastric secretory process?* On the basis of what has been said above, the balance of evidence indicates that histamine is the only truly potent gastric secretory stimulant present in the gastric mucous membrane Since it is established as the most potent gastric gland excitant known, the question of its identity with the hormone involved in normal gastric secretion is one meriting serious consideration On the basis of what is known, a sharp discrepancy is at once manifest—it is generally agreed that atropine will completely inhibit the secretory response of a dog to a meal, and all of the individual phases of normal gastric secretion, whereas numerous investigators have shown that atropine will only attenuate, but not abolish, the response of a dog to a single large dose of histamine This paradox is partially resolved by the finding of Kim (177) that 1 mg of atropine will abolish the response to small doses of histamine, of the order of 0 2–0 3 mg Gray (109) noted that the extent of inhibition of histamine secretion depended on the dosage of histamine and the rate of secretion induced, but that inhibition was never complete, and that atropine inhibition was not complete when applied to a secretory rate somewhat less than that obtaining during the intestinal phase of secretion in the same dogs, the latter being subject to atropine eradication Gray suggested that, if histamine is involved in the normal process of gastric secretion, atropine must inhibit its formation

Crucial proof of the operation of histamine as the gastric hormone might be furnished by the demonstration of two circumstances first, that the histamine titer of the arterial blood is increased after ingestion of a meal, and second, that the humoral response is eradicable by the administration of histaminase MacIntosh (230) attempted to show an increase in blood histamine after feeding, as well as its increased concentration in the gastric juice, and was unable to do so However, his results were likewise negative even after the subcutaneous injection of 1 mg of histamine Ivy, Atkinson, and Bass (153) found no significant reduction in the secretory response to a meal after histaminase administration However, the histaminase was given orally to their human subjects and quite conceivably was unabsorbed, or absorbed in amounts inadequate to be effective Attempts to inhibit the gastric secretory response to histamine by the injection of histamine inhibitors such as benadryl have been in the main unsuccessful (237,246)

To sum up the existing status of the gastrin theory, some comparisons

may be drawn with the evidence in favor of the existence of this hormone as opposed to that in support of an established agent such as secretin In the case of secretin, the following facts are known

(1) There is a specific area of stimulation, the small intestine
(2) The substances (HCl, soap, peptone, etc) which cause the absorption of the hormone from that area, do not act by being themselves absorbed, since the most potent one, HCl, is ineffective when given intravenously
(3) The effect is entirely independent of the innervation
(4) Active extracts can be prepared from the specific area which produce an effect identical with the physiological stimulus

When the gastrin theory is subjected to a similar analysis, it is revealed that

(1) There is no demonstrable specific area of stimulation The evidence favoring the pylorus is inadequate
(2) The substances which excite the area are all active parenterally, though the best evidence indicates that in eliciting the hormone effect they do not act by absorption
(3) The effects have not conclusively been shown to be independent of the innervation
(4) Active extracts can be prepared from any body tissue, and their action on injection is not identical with the normal response

The evidence in favor of a humoral mechanism is conclusive The evidence in favor of a hormone as the humoral agent is much less decisive However, the evidence favoring gastrin as a distinct entity is at present inadequate, although no proof exists that it is identical with histamine

IV. The Salivary Glands

The secretion of the salivary glands is generally known to be under the control of nervous reflexes affecting the secretory cells, the blood flow, and the contractile components of the glands In support of the conception that the nervous element is sufficient in itself, Langley (209) and Malloizel (233) demonstrated that for a period of time after denervation the submaxillary gland will not respond to so potent a stimulus as the application of acid to the mouth Sacks and Kim (291) confirmed their observations, and in addition found that acid extracts of the mucosa of the mouth, pharynx, and tongue failed to stimulate the flow of saliva when injected Several known hormones have been tested for their effect on salivary secretion Secretin concentrates (52,314), pituitrin (312), and insulin (182) have been found to be ineffective as stimulators,

thyroid has been reported to sensitize the secretory nerves (196,262), and epinephrine in large doses will stimulate (7,210), but this effect is merely a duplication of the activity of one of the components of glandular innervation Injection of perfusates from a secreting salivary gland have been reported to stimulate the flow of saliva, whereas perfusates from a resting gland were ineffective (49,50,51,279) This action is now known not to be hormonal, but rather is due to the presence of neuromedins in the perfusate from the active gland

It has been reported from various sources that the salivary glands produce an internal secretion which influences carbohydrate metabolism, either directly or via an action on the pancreas Most of these indicate the presence of an insulin antagonist Several investigators (8,15,27,57) have characterized the effects of bilateral parotidectomy to include hypoglycemia, diminution of induced hyperglycemia, and hypertrophy and hyperplasia of the pancreatic islet tissue Zimmerman and Soskin (358) noted that bilateral ligation of the duct of the dog's parotid gland produced an increased sugar tolerance in the normal animal, but that no such result obtained in a dog after pancreatectomy It has also been stated (86,174) that many or all patients seen with bilateral enlargement of the parotid glands were diabetic On the basis of this apparent evidence that the parotid glands elaborate an anti insulin principle, extracts of the glands have been prepared, the injection of which was stated to produce a hyperglycemia (16,148) and, if long continued, degenerative changes in the islet tissue (16) However, other workers have apparently extracted a blood sugar-lowering substance from the salivary glands (328) and the belief is expressed by some that in the event of deficient insulin production by the pancreas the salivary glands operate to maintain constant the blood sugar level (56,81,284)

In view of the meager evidence presented in the work cited above and the discordant nature of the reports, it is impossible at present to assign to the salivary glands any role in the regulation of carbohydrate metabolism It is certain that if one does exist, it is so minor as to be insignificant

V. Urogastrone

A few years after the discovery of enterogastrone, Sandweiss and co-workers (297,298) reported that commercially prepared extracts of human pregnancy urine containing the chorionic gonadotropic hormone served to prevent or delay the onset of experimentally produced ulcers in dogs The possible mechanism of such protection was investigated by Culmer, Atkinson, and Ivy (40), who found such extracts to inhibit gastric secretion, and it was shown by Gray, Wieczorowski, and Ivy (114) that

the effect was not a characteristic of pregnancy urine since it was also obtainable from extracts of normal female and male urine Confirmation of their findings promptly appeared (99 253) and the presence of an inhibitor reported in the urine of patients with peptic ulcer (99a) pernicious anemia (23 24) and gastric carcinoma (99a) and also in the achlorhydric gastric juice of patients suffering with pernicious anemia or gastric carcinoma (24) It was likewise demonstrated to be present in normal dog urine as well as in human urine (115) The active concentrates as *first prepared contained pyrogenic substances in measurable amounts* which are known to depress gastric secretion (244 339) but a process for the preparation of pyrogenfree active extracts was developed by Gray *et al* (112) and this clearly demonstrated the existence of a gastric inhibitory factor distinct from pyrogen and from the gonadotropins which was given the name urogastrone

The obvious similarity in physiological effect of urogastrone and enterogastrone has prompted investigations with a view to determining whether the two were identical substances or whether urogastrone represented a metabolic or excretory product of enterogastrone as a result of which certain differences between the two have been demonstrated It has been shown that when equivalent doses based on the gastric secretory inhibitory effect are administered the duration of motor inhibition is far less in the case of urogastrone as compared to enterogastrone obtained by the tannic acid process (111) and that the motor inhibitory principle of enterogastrone is destroyed by peptic digestion whereas such treatment does not affect the motor inhibitory potency of urogastrone (142) Several studies have been made to determine whether urogastrone represents an excretory product of enterogastrone The information yielded by these has not led to any definite conclusion on this particular point but has revealed several items of significance Thus it has been shown (41 351) that when dogs were completely enterectomized there was usually a decrease in the urogastrone output in the urine but this does not always take place No decrease was *manifested* when the small intestine was removed from continuity with the alimentary tract but left in the abdomen and when the surgical procedure was limited to exclusion from the intestine of gastric and pancreatic juice and bile the urogastrone output was apparently augmented Furthermore the urogastrone output in human subjects was found to be greater after a fatty meal than during fasting However a fatfree meal caused a similar augmentation (110) The possibility that urogastrone operates by stimulating the release of enterogastrone was ruled out by the work of Schiffrin and Gray (304) who showed urogastrone to be as effective in the enterectomized dog as in the dog with the

small intestine intact In other words, it was effective in animals deprived of their source of enterogastrone

Kaulbersz and co-workers (171) prepared urogastrone concentrates from intact dogs, and from dogs which were ovariectomized, with or without thyroidectomy, or hypophysectomized The urogastrone concentrates from the latter group appeared to stimulate, rather than inhibit, histamine-induced gastric secretion

Urogastrone concentrates have been shown to inhibit the gastric secretory response to histamine in the human (116) Like enterogastrone they fail to inhibit the motility in a vagotomized pouch (143) An inhibition of the stimulating effect of secretin on pancreatic secretion has been noted, and this effect is not destroyed by boiling (128) There is no inhibitory effect exercised on the secretion of saliva or of urine (110)

The effectiveness of urine concentrates in the prophylaxis and treatment of experimentally produced gastrojejunal ulcers in dogs has been investigated by Sandweiss and collaborators They have reported the principle effective against the development of ulcer to be distinct from urogastrone (12,293 295,296) and to manifest the biological effect of producing fibroblastic proliferation, vascularization, and epithelialization of the mucosa of the intestine This factor, designated "anthelone," was stated by Sandweiss (294) to produce protection lasting beyond the duration of therapy in the Mann-Williamson dog, similar to that obtained with enterogastrone (137,151) This factor has been reported absent in the urine of peptic ulcer patients on the basis of the failure of extracts from such a source to protect the Mann Williamson dog (299), and on the basis of finding such extracts to inhibit gastric motility in the gastrostomized dog, a differentiation of the anthelone from the motility-inhibiting principle has been postulated (20)

REFERENCES

1 Abel, J J , and Kuboda, S *J Pharmacol* **13**, 343 (1919)
2 Ågren, G *Skand Arch Physiol* **70**, 10 (1934)
3 Ågren G *ibid* **81**, 234 (1939)
4 Ågren G , and Hammarsten E *J Physiol* **90**, 330 (1937)
5 Ågren G , and Lagerlof H *Acta Med Skand* **90**, 1 (1936)
6 Akim-Peretz, K *Arch Verdauungs-Krankh Stoffwechselpath u Diätetik* **4**, 397 (1898)
7 Alpern, D *Arch ges Physiol Pflugers* **209**, 738 (1925)
8 Aunap E Z *mikroskop-anat Forsch* **24**, 412 (1931)
9 Backman, W Z *klin Med* **40**, 224 (1900)
10 Bauer, A and Uvnas B *Acta Physiol Skand* **8**, 158 (1943)
11 Bayliss, W M , and Starling E H *J Physiol* **28**, 325 (1902)
12 Beaver, D C , Sandweiss D J , Saltzstein, H C , Farbman A A , and Sanders, A W *Am J Clin Path* **12**, 617 (1942)
13 Bekker, N M *Arch sci biol* **2**, 433 (1893)

14 Bernard, C De la Physiologie Général Masson et Cie, Paris, 1872
15 Birnkrant, W B *J Lab Clin. Med* **28**, 1009 (1941)
16 Birnkrant, W B, and Shapiro, R. *ibid* **27**, 510 (1942)
17 Blaubaum, P E *Med J Australia* **1946**, 833
18 Boattini, G *Minerva Med* **2**, 245 (1931)
19 Borchardt, F *Arch ges Physiol Pflügers* **215**, 402 (1926)
20 Bourque, J E, Friedman, M H F, Patterson, T L, and Sandweiss, D J *Gastroenterology* **1**, 1049 (1943)
21 Boyden, E A *Anat Record* **33**, 201 (1926)
22 Braga, J A, and Campos, C M *Gaz clin* **17**, 659 (1919)
23 Brunschwig A, Van Prohaska, J, Clarke, T H, and Kandel, E, *J Clin Invest* **18**, 415 (1939)
24 Brunschwig A, Clarke, T H, Van Prohaska, J, and Schmitz, R. L *Surg Gynecol Obstet* **70**, 25 (1940)
25 Burget, G E *Am J Physiol* **79**, 130 (1926)
26 Butler, D B, Hands, A P, and Ivy, A C *ibid* **139**, 3 (1943)
27 Cahane, M, and Cahane, F *Rev franç endocrinol* **8**, 398 (1933)
28 Camus, L *Compt rend soc biol* **61**, 59 (1906)
29 Cannon, W B Mechanical Factors in Digestion Longmans, Green & Co, New York, 1911
30 Carlson, A J *J Am Med Assoc* **66**, 178 (1916)
31 Carlson, A J Control of Hunger in Health and Disease University of Chicago Press, Chicago, 1916
32 Chang, H T, and Lim, R K S *Chinese J Physiol* **5**, 233 (1931)
33 Chiray, M, and Bolgert, M *Presse méd* **44**, 428 (1936)
34 Chiray, M, Jeandel, A, and Salmon, A *ibid* **38**, 977 (1930)
35 Cohnheim, P Z *klin Med* **52**, 110 (1904)
36 Comfort, M W, and Osterberg A E *Am J Digestive Diseases* **8**, 337 (1941)
37 Comfort, M W, and Osterberg A E *Arch Internal Med* **66**, 688 (1940)
38 Copher, G H, Kodama, S, and Graham, E A *J Exptl Med* **44**, 65 (1926)
39 Crittenden, P J, and Ivy, A C *Am J Physiol* **119**, 724 (1937)
40 Culmer, C U, Atkinson, A J, and Ivy, A C *Endocrinology* **24**, 631 (1939)
41 Culmer, C U, Gray, J S, Adkison J L and Ivy, A C *Science* **91**, 148 (1940)
42 Cunningham R N *Biochem J* **26**, 1083 (1932)
43 Dale, H, and Laidlaw, P P *J Physiol* **41**, 318 (1910)
44 Dale, H, and Laidlaw, P P *ibid* **44**, 11 (1912)
45 De Barbieri, A *Boll soc ital biol sper* **14**, 674 (1939)
46 De Barbieri, A *Rass clin terap e sci affini* **38**, 207 (1939)
47 Delezenne, C, and Frouin, A *Compt rend soc biol* **56**, 319 (1904)
48. Delezenne, C., and Pozerski E. *ibid* **56**, 987 (1904)
49 Demoor, M J *Arch intern physiol* **10**, 377 (1911)
50 Demoor, M J *ibid* **12**, 52 (1912)
51 Demoor, M J *ibid* **13**, 1073 (1913)
52 Deroux, M, Lambert, M, and Meyer, E *Compt rend soc biol* **54**, 1044 (1902)
53 Diamond, J S, and Siegal, S A *Am J Digestive Diseases* **7**, 435 (1940)
54 Diamond, J S, and Siegal, S A *N Y State Med J* **41**, 869 (1941)
55 Diamond, J S, Siegal, S A, Gall, M B, and Karlen, S *Am J Digestive Diseases* **6**, 366 (1939)
56 Dobreff, M *Deut med Wochschr* **62**, 67 (1936)
57 Dobrzianecki, W D, and Michalowski, E *Lyon Chir* **28**, 571 (1931)

58 Dolinski, I L *Arch sci biol Italy* **3**, 399 (1894)
59 Doubilet, H *Gastroenterology*, **7**, 108 (1946)
60 Doubilet, H , and Ivy, A C *Proc Soc Exptl Biol Med* **39**, 129 (1938)
61 Doubilet, H , and Ivy, A C *Am J Physiol* **124**, 379 (1938)
62 Downs, A W *ibid* **52**, 498 (1920)
63 Downs, A W , and Eddy, N B *ibid* **43**, 415 (1917)
64 Downs, A W , and Eddy, N B *ibid* **48**, 192 (1919)
65 Drewyer, G E , and Ivy, A C *Proc Soc Exptl Biol Med* **27**, 186 (1929)
66 Drewyer, G E , and Ivy, A C *Am J Physiol* **94**, 285 (1930)
67 Drewyer, G E , and Ivy, A C *Loc cit* Ivy *Physiol Revs* **14**, 1 (1934)
68 Dubois, C , and Polonovski, M *Compt rend soc biol* **93**, 632 (1925)
69 Edkins, J S *J Physiol* **34**, 133 (1906)
70 Edkins, J S , and Tweedy, M *ibid* **38**, 263 (1909)
71 Enriquez, M M , and Hallion, L *Compt rend soc biol* **55**, 233 (1903)
72 Enriquez, M M , and Hallion, L *ibid* **56**, 322 (1904)
73 Enriquez, M M , and Hallion, L *ibid* **71**, 498 (1911)
74 Ewald, C M , and Boas, M *Arch path Anat Virchow's* **104**, 271 (1886)
75 Fallois, A *Bull acad roy méd Belg* , **1903**, 756
76 Farrell, J I *Am J Physiol* **85**, 672 (1928)
77 Farrell, J I , and Ivy, A C *ibid* **76**, 227 (1926)
78 Farrell, J I , and Ivy, A C *ibid* **78**, 325 (1926)
79 Farrell, J I , and Ivy, A C *ibid* **81**, 475 (1927)
80 Feng, T P , Hou, H C , and Lim, R K S *Chinese J Physiol* **3**, 371 (1929)
81 Feretti, G *Giorn clin med* **17**, 1149 (1936)
82 Ferguson, J , Ivy, A C , and Greengard, H *Am J Physiol* **117**, 701 (1936)
83 Fink, R M *ibid* **139**, 633 (1943)
84 Fink, R M , and Nasset, E S *ibid* **133**, 276 (1941)
85 Fink, R M , and Nasset, E S *ibid* **139**, 626 (1943)
86 Flaum, E *Klin Wochschr* **11**, 1704 (1932)
87 Fleig, C *J physiol path gén* **6**, 32 (1904)
88 Fleig, C *Arch intern physiol* **1**, 287 (1904)
89 Fleig, C *Compt rend soc biol* **55**, 353 (1903)
90 Florey, H W , and Harding, H E *J Path Bact* **39**, 255 (1934)
91 Florey, H W , and Harding, H E *Proc Roy Soc London* **B117**, 68 (1935)
92 Florey, H W , and Harding, H E *Quart J Exptl Physiol* **25**, 329 (1935)
93 Fogelson, S J , and Bachrach, W H *Am J Physiol* **128**, 121 (1939)
94 Frederick, J N , and Greengard, H *Federation Proc* **6**, 105 (1947)
95 Friedenthal, H *Arch sci biol Italy* **12** (1904)
96 Friedenthal, H *Arch Anat Physiol* 126 (1905)
97 Friedman M H F , Haskell, B F , and Snape, W J *Federation Proc* **6**, 107 (1947)
98 Friedman, M H F , and King, E N *ibid* **6**, 107 (1947)
99 Friedman M H F , Recknagel, R O , Sandweiss, D J , and Patterson, T L *Proc Soc Exptl Biol Med* **41**, 509 (1939)
99a Friedman M H F Recknagel, R O , Sandweiss, D J , and Patterson, T L *Anat Record Suppl* **75**, 531 (1939)
100 Friedman, M H F , and Sandweiss, D J *Am J Digestive Diseases* **13**, 108 (1946)
101 Friedman M H F , and Snape, W J *Federation Proc* **4**, 21 (1945)
102 Friedman M H F , and Snape, W J. *ibid* **6**, 109 (1947)

103 Friedman, M H F, and Thomas, J E *ibid* **4**, 22 (1945)
104 Frouin, A, and Lalou, S *Compt rend soc biol* **71**, 189 (1911)
105 Fujimoto, B *Am J Physiol* **47**, 342 (1918)
106 Gavin, G, McHenry, E W and Wilson, M J *J Physiol* **79**, 234 (1933)
107 Gerard, R W, and Still, E U *Am J Physiol* **103**, 232 (1933)
108 Gill, A M *Proc Roy Soc Med* **30**, 517 (1946)
109 Gray, J S *Am J Physiol* **120**, 657 (1937)
110 Gray, J S *Am J Digestive Diseases* **8**, 365 (1941)
111 Gray, J S, Bradley, W B, and Ivy, A C *Am J Physiol* **118**, 463 (1937)
112 Gray, J S, Culmer, C U, Wieczorowski, E, and Adkison I I *Proc Soc Exptl Biol Med* **43**, 225 (1940)
113 Gray, J S, and Wieczorowski, E *ibid* **40**, 324 (1939)
114 Gray, J S, Wieczorowski, E, and Ivy, A C *Science* **89**, 489 (1939)
115 Gray, J S, Wieczorowski, E, and Ivy, A C *Am J Physiol* **126**, 507 (1939)
116 Gray, J S, Wieczorowski, E, and Ivy, A C *Am J Digestive Diseases* **7**, 513 (1940)
117 Greengard, H, Atkinson, A J, and Ivy, A C *Gastroenterology* **6**, 625 (1946)
118 Greengard, H, Dutton D F, and Ivy, A C *Federation Proc* **4**, 27 (1945)
119 Greengard, H, Grossman M, and Ivy, A C *Science* **99**, 351 (1944)
120 Greengard, H, and Hands, A P Unpublished
121 Greengard, H, Hands, A P, Grossman, M, and Ivy, A C *Federation Proc* **2**, 17 (1943)
122 Greengard, H, Hartz, W H, and Ettinger, R E Unpublished
123 Greengard, H, and Ivy, A C *Am J Physiol* **124**, 427 (1938)
124 Greengard, H and Ivy, A C *Federation Proc* **4**, 26 (1945)
125 Greengard, H, and Lindberg, J H Unpublished.
126 Greengard H, Stein, I F, Jr, and Ivy, A C *Am J Physiol* **132**, 305 (1941)
127 Greengard, H, Stein, I F, Jr, and Ivy, A C *ibid* **133**, 121 (1941)
128 Greengard, H, Stein, I F, Jr, and Ivy, A C *ibid* **134**, 245 (1941)
129 Greengard, H, Stein, I F, Jr, and Ivy, A C *ibid* **134**, 733 (1941)
130 Greengard, H, Wolfrom M L, and Ness, R K *Federation Proc* **6**, 115 (1947)
131 Gregory, R A, and Ivy, A C *Quart J Exptl Physiol* **31**, 111 (1941)
132 Grossman, M, Greengard H, Dutton, D F, and Wooley, J *Gastroenterology* **2**, 6 (1944)
133 Grossman, M, Greengard H, Wooley, J, and Ivy, A C *Am J Physiol* **141**, 2 (1944)
134 Hallion, L, and Lequeaux, M *Compt rend soc biol* **61**, 33 (1906)
135 Hammarsten, E, Ågren, G, Hammarsten, A, and Wilander, O *Biochem Z* **264** 275 (1933)
136 Hammarsten, E, Wilander, O, and Ågren G *Acta Med Skand* **68**, 238 (1928)
137 Hands, A P, Greengard, H, Preston, F W, Fauley, G B, and Ivy, A C *Endocrinology* **30** 905 (1942)
138 Harden, A, and Norris, D *J Physiol* **42**, 332 (1911)
139 Harper, A A, and Raper, H S *ibid* **102**, 115 (1943)
140 Harper, A A, and Vass, C C N *ibid* **99**, 415 (1941)
141 Harper, A A, and Mackay, I F S *ibid* **104**, 27P (1945)
142 Harris, S C, and Gray, J S *Federation Proc* **1**, 37 (1942)
143 Harris, S C, Gray, J S, and Ivy, A C Unpublished
144 Heller, J *Arch exptl. Path. Pharmakol.* **145**, 358 (1939)
145 Heller J *ibid* **177**, 127 (1935)

146 Henri, V, and Portier, L *Compt rend soc biol* **54**, 620 (1902)
147 Higgins, G M, and Mann, F C *Am J Physiol* **78**, 339 (1926)
148 Hiki, Y, Akazaki, K, Bann, T, Miyazaki, Y, and Takizawa, N *Trans Soc Path Japon* **22**, 273 (1932)
149 Houssay, B A, and Mollinelli, E A *Compt rend soc biol* **97**, 1032 (1927)
150 Ivy, A C *Physiol Revs* **10**, 282 (1930)
151 Ivy, A C *Gastroenterology* **3**, 433 (1943)
152 Ivy, A C *Am J Surg* **8**, 435 (1929)
153 Ivy, A C, Atkinson, A J, and Bass, V. *Am J Physiol* **132**, 52 (1941)
154 Ivy, A C, Drewyer, G E, and Orndoff, B H *Endocrinology* **14**, 343 (1930)
155 Ivy, A C, and Farrell, J I *Am J Physiol* **74**, 639 (1925)
156 Ivy, A C, and Farrell, J I *ibid* **77**, 474 (1926)
157 Ivy, A C, and Farrell, J I *Proc Soc Exptl Biol Med* **23**, 577 (1925)
158 Ivy, A C, and Farrell, J I *ibid* **23**, 753 (1925)
159 Ivy, A C, and Fisher, N F *Am J Physiol* **67**, 445 (1924)
160 Ivy, A C, Kloster, G, Drewyer, G E, and Lueth, H C *ibid* **95**, 35 (1930)
161 Ivy, A C, Kloster, G, Lueth, H C, and Drewyer, G E *ibid* **91**, 336 (1929)
162 Ivy, A C, Kloster, G, Lueth, H C, and Oldberg, E. *ibid* **85**, 331 (1928)
163 Ivy, A C, and Oldberg, E *ibid* **86**, 599 (1928)
164 Ivy, A C, and Oldberg, E *J Am Med Assoc* **90**, 445 (1928)
165 Ivy, A C, Farrell, J I, and Lueth, H C *Am J Physiol* **82**, 27 (1927)
166 Ivy, A C, Lim, R K S, and McCarthy, J E *Quart J Exptl. Physiol* **15**, 55 (1925)
167 Ivy, A C, Lim, R K S, and McCarthy, J E *Am J Physiol* **67**, 124 (1924)
168 Ivy, A C, and McIlvain, G B *ibid* **67**, 124 (1924)
169 Jung, F T, and Greengard H *ibid* **103**, 275 (1933)
170 Kasanski, N *Jahresber Fortschr Tierchemie* **53**, 552 (1903)
171 Kaulbersz, J, Patterson, T L, Sandweiss, D J, and Saltzstein, H C *Science* **102**, 530 (1946)
172 Keeton, R W, and Koch, F C *Am J Physiol.* **37**, 481 (1915)
173 Keeton, R W, Luckhardt, A B, and Koch, F C *ibid* **51**, 469 (1920)
174 Kenaway, M R *Trans Roy Soc Trop Med Hyg* **31**, 339 (1937)
175 Khizhin, P P *Jahresber Fortschr Tierchemie* **24**, 347 (1894)
176 Kim, M S *Mitt med Akad Kioto* **12**, 731 (1934)
177 Kim, M S *ibid* **17**, 342 (1939)
178 Kim, M S, and Ivy, A C *Am J Physiol* **105**, 220 (1934)
179 King, J F *Arch Internal Med* **42**, 763 (1938)
180 Kiyohira, K I *Compt rend soc biol* **116**, 1166 (1934)
181 Klein, E, and Arnheim, E *Arch Surg* **25**, 433, 442 (1932)
182 Kogen, V M, and Pomrovsky, N *Z ges Exptl Med* **47**, 566 (1925)
183 Kokas, E, and Ludany, G *Arch ges Physiol Pflugers* **232**, 293 (1933)
184 Kokas, E, and Ludany G *Compt rend soc biol* **117**, 972 (1934)
185 Kokas, E, and Ludany, G *Arch ges Physiol Pflugers* **234**, 182 (1934)
186 Kokas, E and Ludany, G *ibid* **234**, 589 (1934)
187 Kokas, E, and Ludany, G *Quart J Exptl Physiol* **28**, 15 (1938)
188 Komarov, S A *Proc Soc Exptl Biol Med* **38**, 514 (1938)
189 Komarov, S A *Am J Physiol* **126**, 559 (1939)
190 Komarov, S A *Rev Can Biol* **1**, 191 (1942)
191 Komarov, S A *ibid* **1**, 377 (1942)
192 Kosaka T, and Lim R K S *Chinese J Physiol* **4**, 213 (1930)

193 Kosaka, T, and Lim, R K S *Proc Soc Exptl Biol Med* **27**, 890 (1930)
194 Kosaka, T, and Lim, R K S *Chinese J Physiol* **7**, 5 (1933)
195 Kosaka, T, Lim, R K S, Ling, S M, and Liu, A C *ibid* **6**, 107 (1932)
196 Krontkovsky, A and Maevsky, W E *Vratchebnoe Delo* 11–15 (1921)
197 Krzywanek, F W *Arch ges Physiol Pflugers* **222**, 435 (1929)
198 La Barre, J *Bull. acad roy méd. Belg* **12**, 620 (1932)
199 La Barre, J, and Houssa, P *Compt rend soc biol* **119**, 538 (1935)
200 La Barre, J, and Ledrut, J *Bull soc chim biol* **15**, 724 (1933)
201 La Barre, J, and Ledrut, J *Compt rend soc biol* **115**, 750 (1934)
202 La Barre, J, and Ledrut, J *Bull soc chim biol* **17**, 459 (1935)
203 La Barre, J, and Ledrut, J *Compt rend soc biol* **115**, 1233 (1934)
204 La Barre, J, and Ledrut, J *Arch intern physiol* **40**, 209 (1934)
205 La Barre, J, and Ledrut J *Compt rend soc biol* **116**, 654 (1934)
206 Lagerlof, H *Quart J Med* **8**, 115 (1939)
207 Lagerlof, H *Acta Med Skand* **120**, 407 (1945)
208 Lang G *Deut Arch klin Med* **78**, 302 (1903)
209 Langley, J N *J Physiol* **6**, 71 (1885)
210 Langley, J N *ibid* **27**, 237 (1901)
211 Laughton N B, and Macallum, A B *Can Med Assoc J* **23**, 348 (1930)
212 Laughton, N B, and Macallum A B *Proc Roy Soc London* B**111**, 37 (1932)
213 Laughton, N B, and Macallum A B *J Biol Chem* **109**, 52 (1935)
214 Lim, R K S *Quart J Exptl Physiol* **13**, 79 (1923)
215 Lim, R K S, Ivy, A C, and McCarthy, J E *ibid* **15**, 19 (1925)
216 Lim, R K S, Ling S M, and Liu, A C *Chinese J Physiol* **8**, 219 (1934)
217 Lim, R K S, Loo, C T, and Liu, A C *ibid* **1**, 51 (1927)
218 Lim, R K S, Chang, H T, Hou H C, and Feng, T P *ibid* **4**, 1 (1930)
219 Lim, R K S, and Necheles, H *Proc Soc Exptl Biol Med* **24**, 197 (1926)
220 Lim, R K S, and Necheles H *Chinese J Physiol* **2**, 415 (1928)
221 Lintvarev, S *Biochem Centr* **1**, 96 (1903)
222 Lobosov, J O *Jahresber Fortschr Tierchemie* **27**, 389 (1896)
223 Loew, E R, Gray J S, and Ivy A C *Am J Physiol* **126**, 270 (1939)
224 Loew, E R, Gray, J S, and Ivy, A C. *ibid* **128**, 298 (1940)
225 Loew, E R, Gray, J S, and Ivy, A C *ibid* **129**, 659 (1940)
226 Lonnqvist, B *Skand Arch Physiol* **18**, 194 (1906)
227 Luckhardt, A B, Keeton, R W, and Koch, F C. *Am J Physiol* **50**, 529 (1920)
228 Lueth, H C, Ivy, A C, and Kloster, G *ibid* **91**, 329 (1929)
229 Lueth, H C, and Kloster, G *ibid* **85**, 389 (1938)
230 MacIntosh, F C *J Physiol* **28**, 87 (1938)
231 Magnus, R *Arch ges Physiol Pflügers* **108**, 1 (1905)
232 Magnus, R *ibid* **122**, 210 (1908)
233 Mallouzel, L *J physiol path gén* **4**, 651 (1902)
234 Matsuo, I *J Physiol* **45**, 447 (1912)
235 Maydell, E *Arch. ges Physiol Pflugers* **150**, 290 (1913)
236 Maydell, E *Physiol Abstracts* **2**, 146 (1917–18)
237 McGavack, T H, Elias, H, and Boyd, L G *Gastroenterology* **6**, 439 (1946)
238 Mellanby, J *J Physiol* **60**, 85 (1925)
239 Mellanby, J *Lancet* **211**, 215 (1926)
240 Mellanby, J *J Physiol* **61**, 38 (1926)
241 Mellanby, J *ibid* **64**, 331 (1928)

242 Mellanby, J *ibid* **66**, 1 (1928)
243 Mellanby, J , and St Huggett, G *ibid* **59**, 122 (1926)
244 Meyer, J , Cohen, S J , and Carlson, A J *Arch Internal Med* **21**, 354 (1918)
245 Meyer, J , Ivy, A C , and McEnery, E T *ibid* **34**, 129 (1924)
246 Moersch, R U , Rivers, A N , and Morlock, C G *Gastroenterology* **7**, 91 (1946)
247 Moore, B , Eadie, E S , and Abram, J H *Biochem J* **1**, 28 (1906)
248 Morel, L , and Terroine, E *Compt rend soc biol* **67**, 36 (1909)
249 Mortimer, B , and Ivy, A C *Am J Physiol* **91**, 220 (1929)
250 Munch-Petersen, J , Ronnow, E , and Uvnäs, B *Acta Physiol Skand* **7**, 289 (1944)
251 Nasset, E S *Am J Physiol* **121**, 481 (1938)
252 Nasset, E S , Pierce, H B , and Murlin, J R *ibid* **111**, 145 (1935)
253 Necheles, H , Hanke, M E , and Fantl, E *Proc Soc Exptl Biol Med* **42**, 618 (1939)
254 Oehme, C , and Wimmers, K *Z ges exptl Med* **38**, 1 (1923)
255 Okada, J *J Physiol* **49**, 457 (1914)
256 Okada, J *ibid* **50**, 42 (1914)
257 Orbeli, L A *Jahresber Fortschr Tierchemie* **36**, 397 (1906)
258 Osborne, S L , and Greengard, H *Am J Physiol* **133**, 404 (1941)
259 Ott, I , and Scott, J C *Proc Soc Exptl Biol Med* **13**, 12 (1915)
260 Philipsborn, H F Jr , Lawrence, G , Gibson, S , and Greengard, H *J Pediat* **26**, 107 (1945)
261 Piontkovski, L *Jahresber Fortschr Tierchemie* **36**, 396 (1906)
262 Poinrovski, N *Vratchebnoe Delo* No 7 (1924)
263 Ponomarew S J Dissertation St Petersburg 1902
264 Popielski, L *Arch ges Physiol Pflugers* **86**, 215 (1902)
265 Popielski, L *Zentr Physiol* **16**, 505 (1902)
266 Popielski, L *Biochem Z* **1**, 225 (1902)
267 Popielski, L *Arch ges Physiol Pflugers* **120**, 451 (1907)
268 Popielski, L *Z Physiol* **16**, 128 (1903)
269 Popielski, L. *Arch ges Physiol Pflugers* **170**, 188 (1907)
270 Popielski, L *ibid* **171**, 239 (1908)
271 Popielski, L *Compt rend soc biol* **72**, 412 (1912)
272 Popielski, L *Z Physiol* **16**, 121 (1902)
273 Popielski, L *Arch ges Physiol Pflugers* **136**, 483 (1909), **138**, 199 (1909), **150**, 1 (1913)
274 Popielski, L. *ibid* **178**, 214 (1920)
275 Popper, H , and Necheles, H *Gastroenterology* **1**, 490 (1943)
276 Portis S A , and Portis, B *J Am Med Assoc* **86**, 836 (1926)
277 Priestley, J T , and Mann, F C *Arch Surg 25, 395 (1932)*
278 Pringle, H *J Physiol* **40**, 42 (1911)
279 Quagliariello, G *Atti accad Med Chim Naples* **74**, 7 (1920)
280 Quigley, J P Zettleman, H , and Ivy, A C *Am J Physiol* **108**, 643 (1934)
281 Razenkov, I P *Arch ges Physiol Pflugers* **223**, 146 (1929)
282 Razenkov, I P *Arch sci biol* **25**, 27 (1925)
283 Razenkov, I P *Arch ges Physiol Pflugers* **226**, 786 (1931)
284 Rerisse A *Rev sud-americana endocrinol immunol quimioterap* **24**, 165 (1941)
285 Risley, E A Raymond, W B , and Barnes, R H *Federation Proc* **6**, 285 (1947)

286 Rogers, J , Fawcett, G G , Rahe, J M , and Beebe, S P *Am J Physiol* **37**, 453 (1915)
287 Rogers, J , Fawcett, G G , Rahe, J M , and Hackett G S *ibid* **39**, 154 (1916)
288 Rogers, J , Rahe, J M , and Ablahadian, E *ibid* **48**, 79 (1919)
289 Rutherford, W *Trans Roy Soc Edinburgh* **29**, 133, (1880)
290 Sacks, J , Ivy, A C , Burgess, J P , and Vandolah, J *Am J Physiol* **101**, 331 (1932)
291 Sacks, J , and Kim, M S *Proc Soc Exptl Biol Med* **28**, 183 (1929)
292 Sandblom, J P , Voegtlin, W L., and Ivy, A C *Am J Physiol* **113**, 175 (1935)
293 Sandweiss, D J *Hebrew Med J* **2**, 182 (1942)
294 Sandweiss, D J *Gastroenterology* **1**, 965 (1943)
295 Sandweiss, D J , and Friedman, M H F *Am J Digestive Diseases* **7**, 50 (1940)
296 Sandweiss, D J , and Friedman, M H F *ibid* **9**, 166 (1942)
297 Sandweiss, D J , Saltzstein, H C , and Farbman, A A *ibid* **5**, 24 (1938)
298 Sandweiss, D J , Saltzstein, H C and Farbman, A. A *ibid* **6**, 16 (1939)
299 Sandweiss, D J , Sugarman, M H , Friedman, M H F , Saltzstein, H C and Farbman, A A *ibid* **8**, 371 (1941)
300 Santos, R. N *Bull soc chim biol* **7**, 1151 (1925)
301 Santos, R N *Endocrinology* **12**, 199 (1928)
302 Savitsch, V V *Russ Physiol J* **4**, 155 (1922)
303 Savitsch, V V , and Zelony, P P *Arch ges Physiol Pflügers* **150**, 128 (1913)
304 Schiffrin, M J , and Gray, J S *Am J Physiol* **137**, 417 (1942)
305 Schiffrin, M J , and Nasset, E S *ibid* **128**, 70 (1939)
306 Serano, C *Rass clin terap e sci affini* **36**, 247 (1937)
307 Shay, H , Gershon Cohen, J , and Fels, S S *Ann Internal Med* **11**, 1563 (1938)
308 Shay, H , Komarov, S A , Fels, S S , Meranze, D , Gruenstein, M , and Siplet H *Gastroenterology* **5**, 43 (1945)
309 Slive, A , Bachrach, W H , and Fogelson, S J *Surg Gynecol Obstet* **70**, 1666 (1940)
310 Smidt, H *Arch klin Chir* **125**, 26 (1923)
311 Sokolov, A. P *Jahresber Fortschr Tierchemie* **34**, 469 (1904)
312 Solem, G O , and Lommen, P A *Am J Physiol* **38**, 339 (1915)
313 Sonnenschein, R. R , and Ivy, A C Unpublished
314 Starling, E H *Lancet* **2**, 501 (1905)
315 Steinberg, M E , Brougher, J C , and Vidgoff, I J *Arch Surg* **15**, 749 (1927)
316 Stepp, W J *Physiol* **43**, 441 (1912)
317 Stepp, W W *Giessen Z biol* **52**, 202 (1914)
318 Still, E U *Am J Physiol* **91**, 405 (1930)
319 Still, E U , and Barlow, O W *ibid* **81**, 341 (1937)
320 Still, E U , McBean, J W , and Reis, F A. *ibid* **99**, 94 (1931)
321 Straus, H , and von Aldor, L *Z diät Phys Therap* **1**, 1117 (1898)
322 Takacs, L *Z ges exptl Med* **57**, 527 (1927)
323 Takacs, L *ibid* **57**, 532 (1927)
324 Takacs, L. *ibid* **60**, 414 (1920)
325 Takacs, L *ibid* **60**, 424 (1928)
326 Takacs, L. *ibid* **62**, 114 (1928)
327 Takacs, L. *ibid* **63**, 553 (1928)

328 Takacs, L *Orvosi Hetilap* **77**, 659 (1933)
329 Tanturi, C A, Ivy, A C, and Greengard, H *Am J Physiol* **120**, 336 (1937)
330 Templeton, R D *loc cit*, Still, *Physiol Revs* **11**, 328 (1931)
331 Tomaszewski, Z *Zentr Physiol* **27**, 630 (1913)
332 Tomaszewski, Z *Arch ges Physiol Pflugers* **171**, 1 (1918)
333 Troller, J Z *klin Med* **38**, 183 (1899)
334 Tschukitscheff, L P *Arch ges Physiol Pflugers* **223**, 251 (1930)
335 Uvnas, B *Acta Physiol Skand* **4**, Suppl XIII (1942)
336 Uvnas, B *Acta Physiol Skand* **6**, 97 (1943)
337 Uvnas, B *ibid* **6**, 117 (1943)
338 Uvnas, B *ibid* **9**, 296 (1945)
339 Vanzant, F R, and Snell, A M *J Clin Investigation* **11**, 647 (1932)
340 Visscher, F E, and Rayman, D R *Federation Proc* **6**, 219 (1947)
341 Voegtlin, W L Unpublished
342 Voegtlin, W L, Greengard, H, and Ivy, A C *Am J Physiol* **110**, 198 (1934)
343 Voegtlin, W L, McEwen, E G, and Ivy, A C *ibid* **103**, 121 (1933)
344 Walsh, E L *ibid* **100**, 594 (1932)
345 Weaver, M M, Luckhardt, A B and Koch, F C *J Am Med Assoc* **87**, 640 (1926)
346 Wells, J A, Gray, J S, and Dragstedt, C A *J Allergy* **13**, 77 (1941)
347 Wertheimer, E and Boulet, L *Compt rend soc biol* **70**, 60 (1911)
348 Wertheimer, E, and LePage, L *J physiol et path gén* **3**, 643 (1901)
349 Wertheimer, E, and LePage, L *ibid* **4**, 1061 (1902)
350 Wick, A N, Pauls F, Irish, I J, and Mackay, E M *Federation Proc* **6**, 226 (1947)
351 Wieczorowski E, Gray, J S, Culmer, C U, and Wells, J A *Am J Physiol* **133**, 490 (1941)
352 Wilander, O, and Ågren, G *Biochem Z* **250**, 489 (1932)
353 Wilhelmj C M, and Finegan, R W *Am J Digestive Diseases* **5**, 372 (1938)
354 Wilhelmj, C M, O Brien, F T, and Hill F C *Am J Physiol* **116**, 685 (1936)
355 Wirschubski *Jahresber Fortschr Tierchemie* **30**, 374 (1900)
356 Wright, R D, Jennings, M A Florey, H W, and Harding, H E *Quart J Exptl Med* **30**, 73 (1940)
357 Zilva, L *J Physiol* **81**, 330 (1904)
358 Zimmerman L M, and Soskin, S *Arch Internal Med* **49**, 663 (1932)
359 Zuelzer, G *Klin Wochschr* **45**, 2065 (1908)
360 Zunz, E, and LaBarre J *Arch intern physiol* **31**, 20 (1929)
361 Zunz E, and LaBarre, J *Compt rend soc biol* **98**, 1435 (1928)

Chapter VII

The Physiology and Chemistry of the Parathyroid Hormone

By ROY O GREEP

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I Introduction

The parathyroid glands were described in 1880 by Sandstrom, but their importance to the health of the organism was not realized until after 1891 when, through Gley's rediscovery of the "external" pair of glands (77), thyroidectomies on humans and dogs were made with the precaution to leave these two small neighboring glandules intact Since the fatal convulsive symptoms which had attended the early attempts at removal of the thyroid were thereby averted, some delineation of the function of the thyroid and parathyroid was already achieved Great interest was aroused in the latter glands when their relationship to calcium metabolism was established in 1908–1909 by MacCallum and Voegtlin (128,129) Despite this exquisite demonstration of the physiological role of the parathyroids, a great amount of work was done in the succeeding years in vain attempts to link the parathyroid in some way with the ability of the body to rid itself of obscure toxins There was a widespread belief that the tetany seen after parathyroidectomy was due to the accumulation of a toxic substance

The endocrine nature of the parathyroids and their real purpose in the body economy was established in 1925 by Collip and co-workers (44–47) They succeeded, in a close competition with several other laboratories, in (1) extracting an active physiological agent from the parathyroid glands of cattle (2) demonstrating conclusively the ability of this agent to restore to well being dogs that were desperately ill from loss of the parathyroid glands, and (3) by the same action, to superimpose a state of hyperparathyroidism on intact dogs This hormone preparation, with some minor modifications in the technique of extraction, satisfied several of the practical clinical requirements which may have contributed to the early falling off in intensive investigation of this glandular product From 1925 to the present progress in parathyroid physiology has gone hand in hand with developments in the much larger field of mineral metabolism On the chemical side, Tweedy and co-workers have continued to explore the properties of the parathyroid hormone with the accepted handicap of not having the hormone in pure form In recent years Ross and Wood (166) have made a notable advance in concentrating the active fraction The extensive clinical investigations by Albright

and collaborators have gone far in elucidating the derangements of mineral metabolism that accompany the various diseases of the parathyroids in man, and have resulted in many improvements in the diagnosis and treatment of these patients

There are many reasons for believing that a revival of interest in the parathyroid field is certain to follow, there is in fact some evidence that it is already under way In keeping with this expectation the intention has been, in this discussion, to provide a guide to the literature and an orientation covering many of the ramifications of the parathyroid subject matter

II Embryology and Histology

The mammalian parathyroid glands are derived embryologically from the third and fourth pharyngeal pouches (parathyroids III and IV, respectively), and the definitive glands are located close to the thyroid gland, the lateral lobes of which are thought to arise in part from the fifth pharyngeal pouch The parathyroids are composed of closely packed polygonal cells arranged sometimes in irregular clumps, or as anastamosing cords, or more rarely in the form of acini with scanty colloid There are occasionally two types of parenchymal cells present, *viz*, (1) the invariably present chief (or principal) cells having unusually pale cytoplasm and containing nuclei with a prominent chromatin network, (2) the oxyphiles larger, and having eosin staining cytoplasmic granules, which are an inconstant component appearing in the human gland only *after childhood and never present in most animals It has not been* possible to ascribe any functional difference to these two types of cells and it is believed that the oxyphiles represent a transitional, probably degenerative, phase of the chief cells The absence of secretory granules in the chief cells is enigmatic in view of the irrefutable physiological evidence that internal secretion occurs Thus far cytoplasmic granules have been found only in the Virginia deer (81) and while these were believed to represent secretory antecedents, Grafflin (82) was not able to find a seasonal variation in the number or characteristics of these granules in correlation with the annual cycle of calcium deposition in antlers

III Anatomy

The parathyroids are commonly four in number and are located along the dorsolateral border of the thyroid as a superior and inferior pair (parathyroids IV and III respectively) of glands There is a great amount of variation in their number and location in animals and in men In twenty five cadavers Heinback (99) found two to six glands each and only 24% of the cases had four glands Most workers have found that

not more than 50% of humans have the supposedly typical four glands In the dog and cat the superior pair of parathyroid glands is embedded in the thyroid, hence to insure complete removal of the parathyroids in these animals it has been a common practice to remove the thyroid also This same arrangement of parathyroids holds for man The rat has only the inferior pair—parathyroid III (80)—and since these are only superficially embedded in the thyroid it is feasible to remove the parathyroid separately (Fig 1)



FIG 1 —A picture showing the relation of the parathyroids to the thyroid gland in the rat and illustrating the technique of parathyroidectomy in this species (From Richter and Eimingham 155)

The parathyroids have a thin connective tissue capsule with delicate trabeculae penetrating the gland Blood is supplied to the parenchyma through sinusoids Cervical sympathetic fibers enter the gland and, as they appear to end mainly in the walls of the vessels, probably play only a vasomotor role

A Accessory Parathyroids

Probably no species is completely free of so-called 'accessory parathyroids which are small clusters of typical parathyroid cells that

become separated from the main glands during embryological development They may be found in the neck region along the carotid artery, and in the anterior and posterior mediastinum, but are most frequently located within the thymus gland Godwin (80) observed fragmentation of the parathyroid anlage in every dog embryo which he examined and concluded that it would be practically impossible to be certain of a complete surgical parathyroidectomy in this species However, in a diligent examination of adult dogs, Reed *et al* (154) found accessory tissue in only one of thirty three animals Hoskins and Chandler (106) made serial sections of the neck regions in embryo, newborn, and adult rat and found accessory parathyroid tissue in only five of sixty five animals examined Swingle and Nicholas (196) were able to correlate survival in their parathyroidectomized cats with the finding of accessory parathyroid tissue The relative frequency of occurrence of these accessory glands is of considerable importance from the experimental viewpoint because they influence to an indeterminable extent the ability of some animals to withstand parathyroidectomy

IV. The Effects of Extirpation of the Parathyroid Glands

A General Effects

The removal of approximately one half of the parathyroid tissue does not produce detectable symptoms, and little (36) or no (165) compensatory hypertrophy of the remaining tissue in rats Even though the total amount of parathyroid tissue is extremely small—0 102 to 0 133 g in man (146)—it thus represents a considerable margin of safety The reactions of different animals to parathyroidectomy vary somewhat but the difference is largely a matter of extent rather than of direction of the changes

The percentage of animals which survive operative removal of the parathyroid is for the dog 0 to 5, cats 20 to 50, rabbits, 67 to 87% (Dragstedt, 53) Survival is measured in terms of animals which under constant conditions either do not develop deficiency symptoms or develop them only transiently It is a common experience that following extirpation of the parathyroids, if life is sustained for several days by palliative procedures mild symptoms of parathyroid deficiency may disappear in a small percentage of animals, thus giving credence to the belief that in the interim accessory parathyroids have assumed sufficient functional importance to sustain the health of the animal without further treatment The survival rates and the much-debated question of whether the parathyroids are necessary for life have become practically meaningless With the extension of our knowledge of the dietary requirements of such

operated animals it is possible to influence survival markedly This does not abrogate or detract from the physiological importance of the parathyroids but rather places them, like the adrenal medulla, in the category of bodily mechanisms the main purpose of which is to enable the animal to meet changing or emergency conditions and to help maintain a mineral homeostasis of the *milieu interne*

The bodily disturbances resulting from ablation of the parathyroids may be divided for the purpose of discussion into (1) neuromuscular symptoms and (2) changes in the chemical composition of the body fluids But it must be kept in mind that these changes are not unrelated

B Neuromuscular Symptoms

The outward signs of parathyroid deficiency as displayed by the dog have been carefully described by MacCallum and Voegtlin (129), Collip (45), and Shelling (182), and are fairly typical of those seen in the more susceptible species including man About sixteen hours after operation the dog becomes restless and refuses food Local and intermittent fibrillary twitchings of muscles appear and these are prodromes of a spectacular succession of disordered and involuntary muscular contractions which will eventually bring the animal to climactic and unrestrained violence (parathyroprival tetany) The twitchings become more general and are followed by tremors, increased muscular tonus, unnatural posture, and uncertain gait The body temperature rises and hyperpnea increases Clonic or tonic spasms appear with great suddenness and eventuate in severe generalized convulsions and laryngeal stridor After a period of one to four hours the severe symptoms abate spontaneously and except for slight depression the dog behaves normally and may eat and play In usually less than twenty four hours these symptoms reappear The interval between attacks decreases and the exhaustion becomes more severe Preterminally the animal lapses into a torpor The limbs are often outstretched in steady and continuous spasticity The immediate cause of death is asphyxiation through spastic contraction of the laryngeal and respiratory smooth musculature or exhaustion Hastings and Murray (96) noted symptoms which point to widespread stimulation of the parasympathetics *e g*, epiphora, enopthalmos, watery nasal secretion, salivation, frequency of urination, and sexual excitement Sympathetic involvement was registered by dilatation of the pupils, partial extension of the nictitating membrane across the cornea, and tachycardia

The spinal cord transection studies of Carlson and Jacobson (38) show that the somatic motor disturbances giving rise to the clonic convulsions originate in the region of the midbrain Although the convulsive movements do not appear caudal to the transection level, hyperirritability and

twitching persist The hyperirritability is a purely peripheral phenomenon The twitches are of spinal origin and are abolished by section of the ventral roots, but not by sectioning the dorsal roots Removal of the cerebral cortex will lessen but not abolish tetany Dogs show such an increased irritability of the phrenic nerve that the diaphragm may twitch with each beat of the heart (due to the action potential) except during inspiratory contraction The sensitivity to painful stimuli is greatly reduced preterminally

Vomiting diarrhea and anorexia are fairly common in acute hypoparathyroidism and each has a significant influence on the development

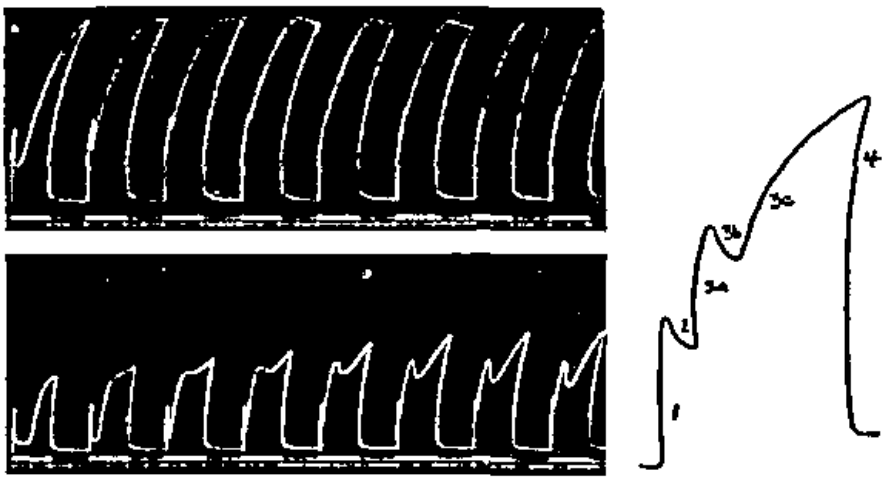


FIG 2 —The effect of removal of the parathyroids on the stages of neuromuscular transmission as exhibited by the soleus muscle of the cat Operation performed 4 days before Indirect stimulation at 500 per sec for 10 sec followed by rest period of 10 sec Top after operation no treatment blood calcium at 6 7 mg % Stages 3b and 3c did not appear Bottom after $CaCl_2$ injection blood calcium at 12 5 mg % Tracing resembles those given by normal cats (From Valenzuela Huidobro and Valdés 212) A code to the various stages is shown to the right of the tracings

of tetany In chronic deficiency peristaltic stasis with resulting constipation may aggravate the tetany Also in protracted parathyroid deficiency, cataract formation is a frequent finding in humans as well as in laboratory animals and has been known to lead to blindness within a few months Changes in the skin and nails of man have been described (123)

Valenzuela *et al* (212) studied the effect of parathyroidectomy on neuromuscular transmission using the response of the soleus muscle of the cat to repeated stimuli at high frequency They found that stages 3b and 3c (Fig 2) as described by Rosenblueth and Cannon (164) did not appear in operated animals, a normal response was obtained after an intravenous injection of calcium chloride

C Changes in Chemical Composition of the Body Fluids

1 *In Dogs*

There is a prompt and steady decline in the calcium content of the blood plasma following removal of the parathyroids This very notable advance in the understanding of the function of the parathyroid glands was made in 1909 by MacCallum and Voegtlin (129) They also demonstrated that the symptoms of tetany in a parathyroidectomized dog were a direct consequence of the lowered blood calcium since the tetany was immediately relieved by intravenous injection of a soluble calcium salt

The calcium in whole blood is almost entirely confined to the plasma About 50% of the calcium is diffusible (nonbound) and the remaining portion is bound as a calcium proteinate or as a part of a negatively charged protein ion The diffusible calcium is either all or nearly all ionized Some workers agree but Schmidt and Greenberg (173) deny that there is a small fraction of diffusible calcium that is bound to some small molecule such as citrate The important aspect is that the calcium proteinate is in equilibrium relation with the ionized calcium McLean and Hastings (137) showed that the ionization of calcium, as measured by the isolated frog heart method, was determined primarily by an equilibrium between the calcium and the plasma protein concentration This is described by the mass law equation

$$\frac{Ca^{++} \times Prot^{--}}{Ca\ Prot} = K = 10^{-2.22} \text{ (at pH 7.35)}$$

They drew up an extremely valuable nomogram which expresses graphically these relations over the range of calcium values that are seen in normal and parathyroidectomized animals

Not all workers are agreed on the extent to which the parathyroids influence the relative proportions of these fractions The evidence, albeit circumstantial, strongly indicates that it is the ionic calcium fraction which is decreased by parathyroid deficiency, but the mechanism whereby this decrease is brought about remains in doubt There is no doubt, however, that a deficiency of calcium ions will produce hyperirritability of the nerves (Loeb, 125, Brink and Bronk, 31) The normal calcium level of the dog (and many other mammals) is 10 5 $\pm$ 1 0 mg per 100 ml serum Symptoms of tetany ordinarily appear when the serum calcium drops below the critical level of 7 mg % and during the convulsive seizures values of 3 5–5 mg % are not uncommon The kidney threshold for the excretion of calcium is only slightly below the normal blood calcium level and during these states of severe hypocalcemia the urine is essentially calciumfree The fecal calcium is reduced or only slightly

elevated The calcium content of muscle and other tissues is unchanged or slightly decreased (198)

The decrease in blood calcium which occurs after removal of the parathyroids is not due to a lowering of the plasma proteins Salvesen and Linder (169) followed the plasma protein values in dogs from the time of parathyroidectomy until violent tetany had developed and found that the total plasma protein concentration remained constant and there was no change in the albumin-globulin ratio but the calcium fell off sharply There is then no primary loss of nondiffusible calcium These results indicate that the calcium deficiency was due to a loss of calcium ions

Greenwald's discovery in 1911 (84) that the urinary elimination of phosphorus was markedly decreased following parathyroidectomy in dogs has been fully substantiated by his later work (86,87) and by many others He also determined that the phosphorus elimination had not merely been shifted toward the feces and, confronted with this definite phosphate retention, he sought unsuccessfully to find a commensurate increase in the serum inorganic phosphate He concluded (85) that the phosphate was stored in the tissues Largely through the work of Salvesen (168), Albright and Ellsworth (6) and Shelling (181), it has been thoroughly established that there is a definite increase in the serum phosphate level that is concomitant with the fall in serum calcium following parathyroidectomy The normal dog has a plasma inorganic phosphate level in the neighborhood of 5 mg % whereas after parathyroidectomy this may rise to 9 mg % or higher Jones (118) and Helfet (100) postulate that, since, in parathyroidectomized animals, calcium ions cannot be mobilized from bone to combine with the increased phosphate to render it inactive and excretable, the calcium ions in the serum are used for this purpose and tetany develops

Hastings and Murray (96) determined, contrary to previous findings, that the pH and the carbon-dioxide-combining power of the blood were not altered in acute hypoparathyroidism nor was there any change in the alkali reserve They expressed the opinion that parathyroprival tetany was not explicable on the basis of an abnormal acid base equilibrium

In acute hypoparathyroidism there is little or no alteration in the amount of serum sodium or potassium It is now clear that calcium and phosphorus metabolism are under the control of the parathyroids and that the adrenal cortex is responsible for the metabolism of sodium and potassium There is no change in the rate of excretion of magnesium in hypoparathyroidism (198)

In summary, the four outstanding metabolic features which characterize the hypoparathyroid state are hypocalcemia and hyperphosphatemia, hypocalciuria and hypophosphaturia

2 *In Other Species*

In parathyroidectomized rats the serum calcium falls to or near the tetany level (7 mg %) but after several weeks it returns to the lower limit of the normal range (9 25–12 5 mg %, 205) These animals rarely show more than muscular twitches with fine tremors of the forepaws and ears unless placed on a low calcium, high-phosphate diet, in which event tetany of varying severity appears in nearly 100% of the animals (Shelling and Ascher, 183, Greep, 89) Cats on the other hand react to parathyroidectomy very much as dogs do, but an occasional animal will die in the acute stage without having exhibited the typical neuromuscular symptoms despite a low blood calcium and high phosphate level Rabbits survive in a high proportion of cases without symptoms, but those which do develop tetany have it in a particularly violent form The blood calcium falls within a few hours after operation and the phosphate remains normal for a while but rises to extraordinary levels in later stages Herbivores in general seem to be less severely affected by removal of the parathyroids than carnivores or omnivores

D Factors Which Modify Parathyroprival Tetany

It is well known that tetany can be induced in normal animals and man by a number of measures, some, such as steatorrhea and rickets, are associated with a fall in the total blood calcium level, and others are not Of the latter, over-ventilation, excess vomiting, and excess sodium carbonate ingestion cause a distinct alkalosis Recalling the clinical features of acute hypoparathyroidism, it will be obvious that the tetany associated with this disease may be subject to considerable modification The rise in body temperature to 105–6°F in parathyroprival tetany leads to hyperventilation which through uncompensated carbon dioxide deficit results in alkalosis Vomiting entails a further loss of acid Warm environments aggravate parathyroprival tetany and cooling has the reverse effect Through the work of Hastings and Murray (96) and McLean and Hastings (137) it is no longer tenable to assume that because of the alkalosis there would be a reduction in ionic calcium of the blood

Injections of neutral or alkaline sodium phosphate lower the blood calcium and produce tetany in normal animals and are very effective in symptomfree parathyroidectomized rats If acid sodium phosphate solution is used tetany does not appear even though the blood calcium level is lowered and the excretion of calcium is increased

Lactose or dextrin feeding helps to prevent tetany after parathyroidectomy The reasonable assumption is made that calcium absorption is facilitated by a more acid fermentation in the gut and perhaps due to the

resulting acidosis less calcium is returned to the gut The production of an acidosis by injection of dilute HCl (214) or ammonium chloride is known to relieve tetany Tetany in man may appear also as a result of steatorrhea Here the absorption of vitamin D from the gut is largely precluded and this contributes to a lowering of the blood calcium

Roby *et al* (161) found that previous vagal section made just above the diaphragm greatly attenuated the neuromuscular symptoms following subsequent thyroparathyroidectomy They noted that a hemocentration appeared at low calcium levels and in a further analysis of this reaction (148) found a rise in serum proteins, a decrease in serum potassium, and no significant change in serum sodium Their dogs persisted in pawing at the head and whimpering which suggested that they may have had severe headache

V. Physiological Activity of the Parathyroid Hormone

The endocrine nature of the parathyroid glands was established by Collip s demonstration in 1925 that an extract of these organs contained

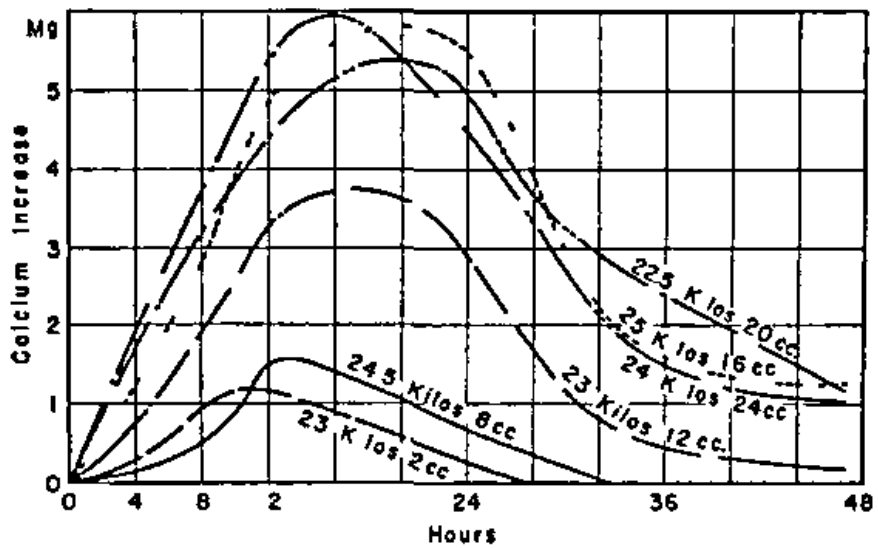


FIG 3 —Constructed curves showing the typical blood calcium response in the intact dog injected with parathyroid hormone and demonstrating the relationship of the response to the dosage (From Coll p 45)

an active principle that would raise the serum calcium level in normal or parathyroidectomized dogs (47) and that there was a fair proportionality between dose and response (45) within the limit of tolerance (see Fig 3) For a given dose the maximum effect on blood calcium was reached in fifteen to twenty four hours if the injection was made subcutaneously or intramuscularly, and in four to eight hours if given intravenously, the hormone apparently is not active by the oral route, contrary to Collip's

early experience The maximum effect is not sustained and the calcium values gradually return to normal The mechanism whereby this response was brought about has not been elucidated Of the many hypotheses so far advanced two continue to merit consideration and these are sufficiently at odds to enliven speculation and stimulate investigation The protagonists of the bone cell theory headed by Selye (178, 179) and by McLean and Bloom (135) believe that the parathyroid hormone controls the rate and direction of mineral exchange between bone and blood by altering the number and metabolic activity of the osteoclasts primarily, and also the osteoblasts, changes in the blood and urine levels of calcium and phosphate are secondary The other school led by Albright maintains that this hormone acts first on phosphorus metabolism in such a way as to increase the renal elimination of phosphate There then follows a lowered blood phosphate which allows the blood calcium to rise and when the kidney threshold is exceeded calcium appears in the urine In consequence of this loss of calcium phosphate the mineral reserves (bones) are attacked through a chemical solution of bone salts As to how this latter step occurs there is a plethora of speculation but little concrete evidence It is hoped that the present interest in phosphatases, especially those found in bone and blood, may help in bringing some enlightenment on this subject

However produced the ultimate effect of parathyroid hormone (PTH) is to raise the serum calcium level In parathyroidectomized animals or man this will restore nerve and muscle irritability to normal and in the intact organism it will suppress nerve irritability through the mobilization of an excess of calcium, presumably calcium ions An increased absorption of calcium from the gut cannot be invoked to account for the hypercalcemia following PTH injections, since neither complete removal of the gastrointestinal tract nor evisceration will prevent the influx of calcium into the blood (192,197) In mineral balance studies on patients, Albright *et al* (7) found no consistent change in the fecal excretion of calcium after PTH therapy They concluded that PTH, unlike AT-10 (dihydrotachysterol) and vitamin D, does not increase the absorption of calcium from the gut

The calcium content of the body tissues shows no significant alteration after PTH administration except for an increase in the kidney and a preterminal, slight increase in heart muscle and liver According to Thomson and Collip (198), the fecal calcium is definitely elevated and there may be a slight increase in fecal phosphorus

It is an accepted fact that PTH causes a striking and prompt increase in the excretion of phosphorus (5,61,88,126) The latter author (Logan) found within one hour after an injection of 34 *U S P* units of PTH in dogs

a distinct increase in phosphate excretion It has also been shown by Tweedy *et al* that the increased excretion of radioactive phosphorus (P^{32}) which is produced by PTH in rats was discernible at one hour and appeared to start immediately after the PTH injection (204) In thyroparathyroidectomized rats PTH produced an increase of twofold or more in the excretion of P^{32} over that of operated controls (206)

Reductions in the serum inorganic phosphorus following PTH administration have been found in rats (126), dogs (35,83), and man (5,6,73) The depression of serum inorganic phosphorus level occurs slowly and is seldom very striking However, readings of 0 5 mg % have been recorded in man as against the normal level of 3–4 mg % In Logan's

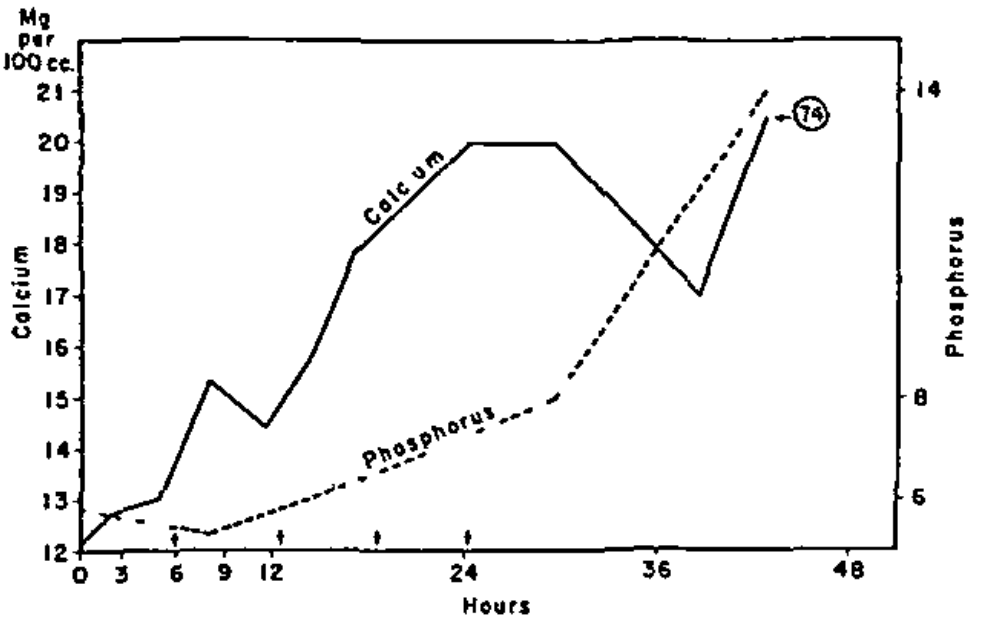


FIG 4 —Showing the blood calcium and blood inorganic phosphorus curves produced in the normal dog subjected to repeated injections of PTH Time of the injections indicated by the arrows (From Collip, 45)

study (126) of the early changes he detected a fall of inorganic phosphate in some of his dogs within one hour after an intravenous PTH injection Since the blood calcium increased in this time whether or not the phosphate fell, he believed that active solution of bone salts may also have taken place in the first hour

The effects of repeating PTH injections at approximately four intervals are cumulative and in dogs lead to death usually in thirty six to forty-eight hours Serum calcium under these conditions rises to a maximum of 20 to 23 mg % and then falls somewhat The serum phosphorus declines during the first several hours, then begins to increase (Fig 4) and with the development of anuria reaches extremely high level

(Esau and Stoland, 63a) The excretion of calcium, phosphate, and nitrogen increases rapidly The ratio of nitrogen to phosphorus decreases indicating that the phosphate is not all coming from endogenous protein metabolism The nonprotein nitrogen and urea content of the blood rise in parallel fashion, thus indicating that toxic nitrogenous compounds are not accumulating Late effects are diarrhea, vomiting, lassitude, anuria muscular atony and coma The blood becomes exceedingly viscous the osmotic pressure increases, and there is dehydration, circulatory failure, and a terminal acidosis At postmortem, calcium deposits are often seen in the kidneys walls of vessels, bronchi, alveoli, and elsewhere indicating renal failure (109 and others) The alimentary tract is congested and there may be blood in the lumen (35,45) The reactions can be duplicated by parenteral administration of excessive amounts of a soluble calcium salt A single acute injection of calcium gluconate causes instant death due to cardiac arrest but when toxic amounts are given slowly the blood becomes viscous and fatal cardiac anoxia ensues (20)



FIG 5 —Bowing of the forelimbs in rat given 15 Collip units of PTH daily for 14 days (From Johnson 116)

Excess dosage with parathyroid hormone causes demineralization of bone In rats, guinea pigs dogs and humans the bones show marked resorption (3,112,113,114,116,117) and replacement with fibrous tissue Spontaneous fractures and bowing of bones have been observed in rats (Fig 5) and dogs, and in hyperparathyroidism of man severe skeletal deformities and fractures have often been described The demineralizing action of PTH is greatly enhanced by partial nephrectomy (144)

Cats are very resistant to intensive PTH treatment and apparently are able to make the necessary excretory adjustments Rabbits show a *hypercalcemia but no late increase in blood phosphorus* *The rabbit is* also peculiar in that it is very difficult to protect it against the appearance of tetany by giving parathyroid hormone, this must be given early and in huge doses to be effective The mouse guinea pig, and fowl are highly resistant to such parathyroid poisoning Kozelka (121) found that rachitic dogs developed only mild tetany after parathyroid ablation and that PTH did not give effective relief whereas intravenous calcium gluconate did

For additional insight on the mode of action of PTH it is important

to know whether the serum calcium level can be raised by PTH in the complete absence of the kidneys If so, then an induced phosphate excretion is not a prerequisite to the calcium raising effect as Albright and co-workers believe Tweedy, McJunkin and co-workers (134,207, 208,209) were unable to obtain a raise in serum calcium with PTH in bilaterally nephrectomized dogs and rats Neufeld and Collip (141) were able to confirm these findings and to show that PTH would not produce an elevation in serum calcium level after renal function had been completely eliminated by various means in rats, cats, and dogs They furthermore were unable to obtain a rise of serum calcium with PTH if by continuous infusion of acid sodium phosphate they prevented the blood phosphate level from falling These results are especially notable in that they led Collip to renounce his earlier adherence to the bone cell theory in favor of the view that the primary action of PTH is to facilitate the excretion of phosphate The latter alternative, however, is not established by these experiments except in the negative sense, for the data do not show that PTH causes an increase in phosphate excretion What these various studies prove is that blood calcium is not increased by PTH if the flow of urine is stopped in otherwise normal dogs Tweedy *et al* (208) asserted that nephrectomy protected dogs from fatal PTH overdosage Ellsworth and Futcher (62) found an increase of 2 to 4 mg % in the serum calcium level of nephrectomized dogs with massive doses (700 units) of PTH, and Stoerk (194) was able to maintain a normal serum calcium level in nephrectomized rats with PTH after the parathyroids had also been removed He considers this as evidence that PTH mobilizes calcium in the absence of the kidney, and maintains with seeming justification that with nephrectomized animals it is not to be expected that PTH will easily superimpose a calcium increment upon the normal calcium level because of the pyramiding amount of phosphate being retained It has also been found that calciferol, like PTH, will mobilize calcium in nephrectomized-parathyroidectomized rats (210), providing a normal blood Ca/P ratio is maintained by controlled mineral intake However, Tweedy *et al* believe that the response is mediated, not by mobilization of calcium from bone, but by restricting the fecal excretion of calcium Monahan and Freeman (138) found that the serum calcium of nephrectomized dogs fell about 50% following removal of the parathyroids

Studies on the relation of the parathyroids to the renal clearance of phosphate and creatine have yielded opposing results Fay *et al* (64) found these clearances were not affected over an extreme range of parathyroid function Logan (126) found no change in the urinary excretion of creatine or creatinine after PTH injections until probable kidney

damage had occurred Harrison and Harrison (97,98), on the other hand, noted that the ratio of maximum tubular resorption of phosphate was decreased by PTH and that the serum inorganic phosphorus was thereby reduced An induced acidosis, however, produced an entirely similar tubular effect That parathyroidectomy reduces the renal excretion of phosphate through a purely renal effect is strongly indicated by the cross-circulation technique employed by Brull and Carbonesco (32)

It is obvious that considerable evidence has been aligned for and against a primary renal action in the parathyroid regulation of mineral metabolism On the basis of this evidence, however, it seems reasonably clear that the ability of PTH to mobilize calcium is greatly impaired in the absence of the kidney A true hypercalcemia has never been produced in such animals It should be kept in mind that many progressive adverse biochemical changes occur in the body when the kidneys cease to function (58,215)

Aside from the observations on the blood calcium level, osteoclast proliferation and osseous resorption have been noted in nephrectomized rats after PTH treatment (48,110,179,194) The fact that nephrectomy alone produced changes in the same direction, although less pronounced, makes it difficult to evaluate this reaction accurately Removal of the parathyroids does, however, prevent bone resorption following nephrectomy (179)

The evidence favoring the bone cell theory of parathyroid function cannot be lightly dismissed It might be suspected from the above discussion that the shifts in blood levels and excretion rates of calcium and phosphate occur more rapidly after PTH administration than could be accounted for on the basis of a histological change in the bone tissue On the contrary these changes appear to coincide quite well McLean and Bloom (135) found an extensive proliferation of osteoclasts and widespread destruction of osteoblasts at six hours after injection of a massive dose of PTH in growing rats At twelve hours the changes were conspicuous and a picture of osteitis fibrosa generalisata was already present at twenty-four hours In a further histological analysis of the mobilization of bone salts in parathyroid treated rats and puppies, these authors (136) were able to demonstrate in sections of undecalcified bone the passage of bone salt from disintegrating trabeculae to the venules of the marrow The bone salt and organic matrix were resorbed simultaneously under the local influence of osteoclasts The bone salts in particulate form and in aggregates of crystals were partly free and partly in the macrophages of the marrow in the spongiosa The osteoclasts were not themselves phagocytic but were often seen to be surrounded and obscured by crystals removed through local cellular action (lacunar

resorption) The calcium phosphate in the macrophages may have been in the form of a colloidal calcium phosphate Gersh (71,72) found that colloidal calcium phosphate introduced into the blood stream was taken up by the macrophages of the liver and spleen A similar phenomenom was seen when rats were injected with parathyroid extract and simultaneously given calcium or phosphate ions in adequate amounts to exceed the solubility product of insoluble calcium phosphate McLean and Bloom (136) have also made the very suggestive preliminary observation that the resorption of bone, under the influence of parathyroid hormone, continued long after the plasma and presumably the tissue fluids had become supersaturated with the salt Herein, however, lies a weakness of the bone cell theory Since both calcium and phosphate are resorbed one should presumably find a hyperphosphatemia as well as a hypercalcemia but the fact is that a *hypo*phosphatemia develops in hyperparathyroidism

After Pugsley (151) found that the increased blood and urine calcium, in rats receiving daily injections of PTH, returned to normal values within ten days, Pugsley and Selye (153) were able to show that this event coincided with the disappearance of osteoclasts, reappearance of osteoblasts, and the resumption of bone deposition The question of the possible development of an immunity to the extract at this time has been considered but there is no evidence available on this point

It is regrettable that the morphological and chemical changes produced by PTH have thus far almost invariably been pursued independently The necessity of integrating these approaches is obvious and the lack of such collaboration has already amassed a backlog of futile effort

A Miscellaneous Effects of Parathyroid Hormone

Parathyroid hormone has a distinct diuretic action and has been used occasionally in the management of nephritis Shelling *et al* (184) showed that dehydration was an important aspect of parathyroid poisoning and protection was afforded by the simple expedient of replacing the fluid and electrolytes that were lost

The concentration of serum magnesium is decreased in hyperparathyroidism (34) while the rate of excretion remains at the normal level (199) or is somewhat increased (126)

In dogs the volume of gastric juice and total gastric acidity is lessened by PTH (14,172) The effectiveness of PTH in mobilizing calcium is greatly diminished in Eck fistula dogs (124) Gastric motility diminishes only after severe calcemia is established (163) PTH has no significant effect on the following basal metabolic rate (191), blood pressure, blood

protein concentration, and blood chlorides (198) There is an increased excretion of calcium in the bile in animals receiving large doses of PTH (120)

Cataracts are often seen in parathyroidectomized animals and in man with idiopathic or operative hypoparathyroidism of long standing The view that parathyroid hormone therapy is more apt to prevent the appearance of cataract than other blood calcium raising measures has been abandoned, as has the possibility that senile cataract may indicate failing parathyroid function (3)

To summarize the histological and chemical changes in the body which result from an excess of the parathyroid hormone have been fairly thoroughly investigated but a great amount of work remains to be done before we shall arrive at a clear understanding of how these several phenomena are brought about and in what order Possibly too much emphasis has been placed on what event is primary It would not be surprising if it should turn out that the parathyroid hormone has more than one focal action, *i e*, it may directly stimulate the proliferation of osteoclasts on the one hand and at the same time directly increase the urinary elimination of phosphate

On the metabolic side one finds in hyperparathyroidism the reverse of the changes seen in hypoparathyroidism Calcium and phosphorus excretion is increased, the calcium content of the blood plasma is elevated and the blood phosphate drops gradually to a subnormal level

VI. Extraction, Purification, and Some Chemical Characteristics of the Parathyroid Hormone

The raw material used in extraction procedures has consisted of fresh frozen, defatted parathyroid glands (bovine) or of acetone-dessicated powdered glands The initial extraction procedure has in all instances (44,94,166 202) consisted of boiling the glandular tissue in dilute acid Three to 5% HCl has been widely used but Allardyce (8) has shown that about 1 5% HCl is optimal The active agent is not removed by extraction with neutral or alkaline aqueous and alcoholic solutions whereas acid aqueous and alcoholic extracts contain the active agent (94) The hormone is apparently destroyed by enzymes during extraction unless hot acid solutions are used

Much inactive material can be removed by adding alcohol to 80% by volume at pH 4 or by making the extract alkaline with NaOH, to dissolve suspended material, and subsequently lowering the pH to 5 5–5 6 with HCl An active fraction may then be removed from solution with ether, trichloroacetic acid or by the familiar salting out procedures These

ether and trichloroacetic acid precipitates can be dried and washed with acetone, ether, or chloroform and are usable as a crude extract Tweedy finds that resuspension in four to five volumes of acid alcohol and reprecipitation with ether yields a product having better solubility in the aqueous injection media Collip extracts the salted-out fraction in weak alkali, centrifuges, and, with addition of acid to the supernatant, he precipitates the active material at pH 4 8 (so-called "isoelectric point") This precipitate, dissolved in HCl solution at pH 3, is put through a Berkefeld filter and standardized for use

Others who have prepared extracts of this gland are Hjort *et al* (104) and Fisher *et al* (65) The crystalline product which Berman (21) prepared and which he claimed would raise the blood calcium in rabbits is regarded with skepticism in view of the erratic behavior of rabbits to injections of parathyroid extracts of proven activity

Ross and Wood (166) suspended a 50 g aliquot of their original extract (N content 11–13%) in 400 ml water and added ammonium hydroxide to pH 8 After adding an equal volume of 2 5 *M* ammonium sulfate the pH was lowered to 5 9–6 0 with molar H_2SO_4 The heavy precipitate was suspended in 250 ml water, dilute ammonium hydroxide added to pH 8, and 2 5 *M* ammonium sulfate was added to bring the suspension to 1 25 *M* concentration A precipitate came down rather sharply at pH 5 9–6 0 This last step was repeated three times and the final precipitate suspended in 150 ml of water, dialyzed in revolving cellophane bags until nearly sulfatefree and put into solution by adding dilute HCl to pH 3 5 This clear brown solution contained on the average 473 mg N with an average total activity of 49,500 *U S P* units (see Section VIII) This preparation was further purified by precipitation with benzoic acid from which the active material was separated by extraction with ether The ether-insoluble residue was dissolved in dilute acid, dialyzed, and adjusted to pH 3 5 This material has a total nitrogen content of 12 6–13 1% and an activity rating of approximately 300 *U S.P* units per mg nitrogen

That the parathyroid hormone is of protein nature seems fully substantiated by the following observations

(1) The xanthroproteic, Millon, biuret, ninhydrin, and Hopkins Cole tests for protein are all positive

(2) The activity of the hormone is destroyed by pepsin and trypsin

(3) The hormone is precipitated by the ordinary protein reagents

(4) Alkaline or acid hydrolysis causes inactivation With gradual acid hydrolysis the increase in free amino nitrogen is closely related to the loss of physiological activity (207)

(5) The ultraviolet absorption spectrum (166) is almost identical with that of some other substances known to be protein

It is not established that the protein in the extract is the hormone, but the general belief that this is true is strengthened by the absence of any evidence on which to base a contrary view and by the following supportive findings (45,166,202)

(1) The activity is rapidly destroyed by proteolytic enzymes

(2) The chemical composition and activity are unaltered by repeated isoelectric precipitation

(3) Small polar groups are not separated from the hormone by electrodialysis

(4) The ultraviolet absorption spectrum shows no indication of the presence of chromophoric prosthetic groups

(5) Adsorption on and elution from permutite does not result in a concentration of the activity

There is no question that the best preparations are inhomogeneous The fact that the isoelectric points vary from preparation to preparation indicates the presence of contaminating inert protein matter Furthermore in a sample subjected to ultracentrifugation by Ross and Wood (166) two components could be identified, one with molecular weight of 500,000–1 000,000, another (65% of the protein and over 50% of the activity) of molecular weight 15 000–25,000 It is also pertinent in this connection that some activity is lost on dialysis against running water (166) Thomson and Collip (198) state that the hormone is not dialyzable through collodion The behavior of the purified preparations in the Tiselius electrophoresis apparatus has not been determined (see addendum pp 293,294)

The hormone is not of the nature of a glucoprotein as is indicated by the fact that the Molisch test for carbohydrate was negative (45,166) The orcinol-HCl test for pentose was likewise negative

VII Stability, Solubility, and Other Characteristics of the Parathyroid Hormone

A Stability

The parathyroid glands can be stored for at least a year in a dry state or as frozen fresh glands without apparent loss of activity The activity of the extracted hormone is best retained in slightly acid (*circa* pH 3–5) media and is slowly lost on standing in neutral or alkaline solution The hormone is stable to treatment with mineral acids and can be safely boiled for at least an hour in concentrations of HCl not exceeding about 5% The activity is completely lost after boiling for one hour in either 10% HCl or 5% NaOH (46)

B Solubility

Collip's best preparations precipitate at pH 4 8 and redissolve in either more alkaline or acid solutions Allardyce (8) finds that the hormone precipitates at pH 4 8 only if this is approached from the acid side, approached from the alkaline side, the precipitate forms at pH 6 Tweedy and Torigne (211) give the isoelectric point as 5 8 The more active preparation of Ross and Wood (166) precipitates at 4 5–5 from the acid side and does not redissolve until extremely alkaline solutions are reached Re-solution occurs at pH 5 from the alkaline side

The active fraction is soluble in water, saline, aqueous alcohol, 94% acetic acid, concentrated warm phenol, or orthocresol, and in warm 50% glycerol It is insoluble in absolute ethyl, methyl, or butyl alcohol, ether, benzene, pyridine, anhydrous acetic acid, methyl salicylate, and carbon tetrachloride (202)

C Inactivation

Some information as to the composition of the hormone and the importance of specific radicals or groups to physiological activity has been gained from the inactivation and reactivation studies in Tweedy's laboratory Activity is destroyed by formaldehyde, acid ethyl and acid methyl alcohol, strong alkali, nitrous acid (one hour, 36 4% deamination), hydrogen peroxide, and potassium permanganate Partial reactivation was achieved after the formaldehyde and acid alcohol inactivation The activity was not destroyed by H_2S, sodium sulfite, sodium amalgam, catalytic hydrogenation, or by reduction with sodium in liquid ammonia (a powerful reducing agent) Wood and Ross (216) produced inactivation with ketene and the activity was not restored on hydrolysis of the O acetyl residues Although these observations cannot be strictly interpreted because of the impure nature of the extracts and lack of information about the hormone molecule they do suggest that (1) the hormone is stable to reducing agents and unstable to oxidizing agents, (2) amino groups are essential to the activity of the molecule, (3) disulfide linkages are not present

With acid hydrolysis (boiling in 0 05 *N* HCl up to seventeen hours) the loss in potency parallels the increase in free amino (207) nitrogen Interest in the nitrogen-containing groups was increased by the experience of Tweedy that variability in potency could not be attributed to variation in the nitrogen partition as determined by the Thimann procedure (177) Collip's preparation contains a trace of sulfur (198) and that of Tweedy, Bell, and Vicens-Rios (203) has 0 20%, which they could not identify as cystine sulfur

Parathyroid hormone has many properties which indicate a similarity to insulin It has not been possible, however, to apply the procedures of Abel and co-workers (1) for the final concentration and crystallization of insulin successfully to parathyroid hormone A further difference in these substances is that the activity of insulin is destroyed by reducing agents (115) whereas PTH is extraordinarily resistant in this regard

VIII Yield and Activity

The average yield by Tweedy's method of extraction is approximately 0 44% calculated on the basis of wet glandular weight, or 4–5% calculated on a dry weight (protein) basis Ross and Wood (166) obtained a yield of roughly 40% after benzoate absorption calculated on the basis of their original crude fraction as starting material The yield varies greatly from preparation to preparation using the same technique, and between different extractive techniques The yields as given are regarded as unpredictably high and imply the presence of inert protein The Collip and Clark preparation (46) has activity of 110 *U S P* units per mg N, that of Ross and Wood (166), 300 *U S P* units on the same basis The Tweedy preparation has an activity of about half that of the Collip and Clark preparation as calculated by Ross and Wood The nitrogen content as given by Thomson and Collip is 15 5%, by Tweedy 14 74, and by Ross and Wood 12 6–13 1%

The *U S Pharmacopoeia* XIII unit is defined as 0 01 of the amount of extract required to raise the serum calcium of not less than ten dogs weighing 8–16 kilos, an average of 1 mg % within 16–18 hours after subcutaneous injection The Collip unit is 0 01 of the amount which will produce an average increase of 5 mg % in the serum calcium of normal dogs of about 20 kg in 15 hours after subcutaneous or intramuscular injections The Hanson unit (95) is 0 01 of the amount required to produce a 1 mg rise in serum calcium of a parathyroidectomized dog within 6 hours

IX Assay Methods

It is universally agreed that none of the methods thus far proposed for assaying parathyroid hormone is satisfactory in terms of accuracy, simplicity, and economy The deviations in serum calcium level in normal or parathyroidectomized dogs as used by the early workers are standard procedures (45) Thomson and Collip (198) maintain that there is no essential difference in the response of normal and operated dogs Hanson (95) feels that the response of the latter is less variable Sex is apparently not a factor The two variables which influence the response most markedly are body weight and individual sensitivity

Ross and Wood (166) select dogs of approximately the same weight and disregard the weight factor The individual sensitivity can be dealt with by using the same animal successively for a comparison between preparations, or by relating the response in a given animal to that produced by a standard sample While attention to the diet of test animals would seem an important consideration, it has been generally disregarded, as has the interval between tests on a given dog Ross and Wood (166) suggest the use of six to ten dogs, but Bliss and Rose (22) by statistical analysis, find that this number is inadequate to establish a standard deviation of 10% The age of the dog is also important, Collip (45) has found that young dogs are more sensitive than old ones The assay is also complicated by the fact that serum calcium changes appear to be more easily elicited near the normal calcium level in normal dogs than, for example, above 16 mg % Assays involving blood calcium in rabbits (93) and rats (201), and urinary calcium excretion of rats (59) offer little promise Gellhorn (70) recommends using the increased height of muscular contractions that PTH will produce when added to the perfusing fluids in a frog leg preparation

X. The Parathyroids in Relation to Other Endocrine Glands

A Pituitary

Several authors claim to have demonstrated increased mitotic activity, hypertrophy, or an increased functional activity of the parathyroids after injection of various crude extracts of the anterior lobe of the pituitary (24,91,102, and others) The slight elevations in serum calcium values which have been obtained in dogs, cats, and guinea pigs after pituitary treatment (10 67,68) are of dubious significance Snyder and Tweedy (190) used the same pituitary preparation as Friedgood (67) and found no change in the serum calcium or inorganic phosphorus in rats Campbell and Turner (36) injected several species with massive doses of several types of pituitary preparations and found no change in the weight or mitotic index of the parathyroids

Houssay and Sammartino (108) claim to have observed degenerative lesions in the parathyroids of 66% of their hypophysectomized dogs In a careful histological examination of the parathyroids from monkeys that had been hypophysectomized in P E Smith's laboratory, Baker (15) found no significant variation from the glands of intact monkeys Smith (189) briefly mentioned that the parathyroids shared in the atrophic changes seen in rats after pituitary oblation Carnes *et al* (39), however, found that hypophysectomized rats were able to maintain normal serum calcium and phosphorus levels even when under the stress

of a low-calcium diet, with both the pituitary and parathyroids out, the rats reacted the same as parathyroidectomized animals The response of *Xenopus laevis* to PTH was not impaired by hypophysectomy as measured by the fall in plasma inorganic phosphate (176) Albright (3) states that in hypopituitarism one does not find clinical evidence of functional deficiency of the parathyroids On the other hand, parathyroid adenomas are sometimes associated with pituitary tumors (90)

B Gonads

A sex difference in the relative weight of the parathyroids has been found only in the rat (23,36), the brown leghorn (119), and in man (75,146), the relatively heavier glands were found in the female The parathyroid glands of nulliparous women are as large or larger than those of multipara according to the data of Pappenheimer and Wilens (146), thus showing that in this species pregnancy is not responsible for the sex difference Gonadectomy apparently has no effect on the parathyroids (36,143) The intense calcemia produced in pigeons by estrogen is not mediated by the parathyroids since the effect is obtained in the absence of these glands (159,160) Campbell and Turner (36) found no increase in mitoses in the parathyroids of chicks treated with estrogen Androgens do not influence the calcium level of birds (132,159) Nathanson *et al* (140) thought testosterone stimulated mitotic activity in the parathyroids of female rats, but this observation was not confirmed by Campbell and Turner (36)

C Adrenals

It has been claimed that normal rats show a greater calcemia and calciuria after PTH injection than do adrenalectomized rats maintained on salt (152), also that removal of the adrenals from seventeen-day rat embryos results in enlargement of the fetal parathyroids at normal term (200)

D Thyroid

The absence of the thyroid does not modify the response of rats to PTH (179) nor does hyperthyroidism in dogs (127) In man hyperthyroidism leads to a striking increase in the urinary excretion of calcium and phosphorus without altering the blood levels, as shown by Aub *et al* (13) They also found x-ray evidence of bone resorption after prolonged hyperthyroidism The calcium excretion in myxedematous patients was markedly less than in normal individuals Logan *et al* (127) found that thyroid treatment did not increase the calcium excretion in thyroparathyroidectomized dogs As they point out, these negative

findings are not conclusive because of the initially low blood and urine calcium Nevertheless the experiment suggests that the effect of the thyroid on calcium metabolism may be mediated through the parathyroids

XI The Bearing of Dietary Mineral Intake, Pregnancy, Lactation, and Renal Inadequacy on the Regulation of Size and Functional Activity of the Parathyroids

The importance of understanding the circumstances which will bring about an increase or a reduction in gland size is well recognized It is a general truism that endocrine organs undergo a compensatory adaptation to the demands made upon them The observation of Rosof (165) that in rats *the parathyroids do not undergo compensatory hypertrophy* following partial parathyroidectomy was not crucial in that numerous isolated observations have shown that such animals have no detectable physiological parathyroid deficiency It is generally accepted, however, that the parathyroids undergo hypertrophy during pregnancy lactation, rickets, and renal insufficiency The larger objective of a number of studies has been to find a common denominator to this stimulatory reaction It seems fairly certain that the parathyroids are not regulated by the hypophysis, nor does it appear at present that they are under the direct or indirect control of any other endocrine gland There is likewise no evidence that the parathyroids are regulated by a nervous mechanism The parathyroids function perfectly as autoplastic grafts with no innervation (196) Dragstedt (54) stimulated the cervical sympathetics for hours without altering the blood calcium or phosphorus levels It appears that the parathyroids are responsive to and are regulated by their chemical environment much as the islets of the pancreas are The problem as it confronts us now is to determine whether this gland is influenced by alterations in calcium or phosphorus concentrations, or both, in the body fluids A refinement of the problem will be to ascertain the relative importance of the ionized versus the unionized fraction of these minerals

A Mineral Intake

That a low calcium intake will result in parathyroid enlargement has been seen many times (40,50,92,131) Indeed it is very widely held on circumstantial evidence that a low blood calcium is the normal stimulus to secretory activity, and this opinion need not be invalidated if it does not appear to hold in certain extreme conditions such as oriental osteomalacia Patt and Luckhardt (147) obtained clear cut physiological evidence that the parathyroids pour out calcium raising hormone when they are perfused with calciumfree blood but do not do so when

perfused with normal blood Only the perfusate of calcium-deficient blood had the ability to raise the serum calcium when administered to normal dogs

The ionic serum calcium and the inorganic phosphorus are to a large degree interrelated, so that a low level of one affords an optimal circumstance for an increase of the other and *vice versa*, providing the normal regulatory mechanisms are functioning It may be noted incidentally that an advantage of this mechanism for the organism is to prevent the

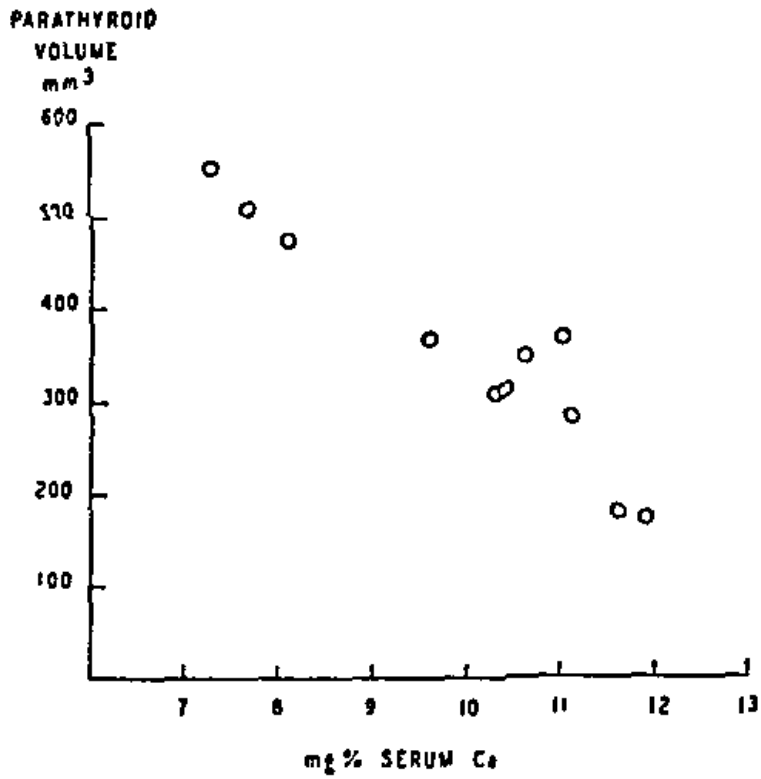


FIG 6 —The relation of the volume of the parathyroids to the blood level of calcium as maintained through controlled intake of calcium and phosphorus (From Stoerk and Carnes, 195)

calcium and phosphate ions from exceeding their solubility product (For a critical review, see Schmidt and Greenberg, 173) Consequently it is not simple to make an evaluation of the relative importance of low blood calcium since it is so often associated with a high phosphate level The point is emphasized by a recent series of papers by Carnes and co workers (40) Using the Steenbock stock ration in conjunction with added minerals, they first claimed that parathyroid enlargement was nearly proportional to added phosphates and they challenged the conclusion of Ham *et al* (92) that calcium was the important element in regulating the size of this gland In a recent continuation of this study

(195), an improved stock ration was used to avoid the complication of general ill-health, and parathyroid enlargement (volume measurement) was then shown to be almost perfectly correlated with the serum calcium level in a series of rats given widely different dietary calcium-phosphorus ratios (Fig 6 and Table I) and different absolute amounts of calcium. These data are clear-cut and appear to be decisive in respect to the influence of calcium as against phosphorus in causing parathyroid enlargement.

TABLE I

THE EFFECT OF THE DIETARY Ca/P RATIO AND OF THE ABSOLUTE CALCIUM AND PHOSPHORUS INTAKE ON THE BLOOD LEVELS OF THESE ELEMENTS AND THEIR RELATIONSHIP TO THE CHANGE IN PARATHYROID VOLUME (195)

group	Diet	% Dietary		Dietary Ca/P ratio	Body weight (g)		Parathyroid volume (mm³)	Serum (mg %)		Serum CA/PO₄ ratio	Bone ash %	No. rats
		Ca	P		Initial	Final		Ca	PO₄			
1	F-908-A	1 053	07	15 1	197	246	173	11 9	3 4	3 5	60 2[1]	10
2	E-908	615	07	8 8	194	225	181	11 6	2 5	4 6	[1]	5
3	F-918-A	2 053	1 17	1 8	163	239	311	10 3	7 3	1 4		9
4	F 918	1 053	62	1 7	164	248	313	10 4	8 2	1 3		8
5	F-918	1 053	62	1 7	201	269	283	11 1	7 1	1 6	64 0	6
6	E-918	1 015	62	1 6	199	251	348	10 6			62 4	6
7	E-907	615	47	1 3	178	241	369	11 0			63 1	5
8	F-913	053	07	8	207	269	371	9 6	4 6	2 1	61 0[1]	6
9	F-909	053	47	11	202	252	475	8 1	7 3	1 1	56 0[1]	5
10	E-909	015	47	03	178	188	508	7 7			59 2[1]	6
11	E 909	015	47	03	212	223	551	7 3				6

[1] Rickets present

Albright (3) maintains that it is a level of serum calcium ions below normal, however produced, that stimulates the parathyroids Stoerk and Carnes (195) found, however, that there was no significant deviation in plasma protein concentration among a sampling of their groups and, since the ionization of calcium is related to this factor (137), it is probable that the marked change in parathyroid volume was not determined solely by the extent to which the calcium proteinate was ionized. In these connections it is interesting to note that in osteomalacia (133) and some forms of experimental rickets, tetany does not appear even though the total serum calcium may have fallen to extreme levels, and one is forced to assume that the ionic calcium has remained above the tetany level through the intermediation of the parathyroids

A low plasma phosphate level is not a stimulus to parathyroid hyperplasia even though the mineral derangement is so perverted that experimental rickets appears (40,92) High phosphate levels (56,100,149,171), on the other hand, have often been associated with parathyroid enlargement, and at some risk of oversimplification, we may infer that this was indirectly due to depression of the blood calcium level Likewise the parathyroid hyperfunction which Baumann and Sprinson (18) saw in rabbits fed a low calcium, high phosphorus diet should be attributed to the low calcium Inanition also causes the parathyroids to enlarge (111) and while the *modus operandi* is not at hand it may again be surmised that low calcium intake is responsible

Just as low blood calcium is associated in some causative manner with parathyroid enlargement and secretory stimulation, a state of hypercalcemia acts as a depressant to parathyroid function and if maintained will cause a slight involution of the gland Carnes *et al* (40) noticed a diminution of parathyroid volume in rats on a low phosphate, high-calcium diet The diminution was accentuated when vitamin D was added Involution of the parathyroids has also been found in rats exhibiting hypercalcemia due either to excess quantities of vitamin D or to rather toxic doses of PTH Jaffe and Bodansky (112) state that the parathyroids in their PTH-treated dogs appeared to be $\frac{1}{2}$ to $\frac{2}{3}$ normal size Pappenheimer and Johnson (145), on the contrary, found no decrease in parathyroid volume in rats treated with PTH nor did this substance prevent the hyperplasia which follows partial nephrectomy It is possible, but there is no evidence, that the PTH *per se* might contribute to parathyroid involution No clue can be taken from the fact that the thyroids and the adrenal cortices atrophy when their respective secretions are injected for the reason that these effects are mediated by the anterior lobe of the pituitary whereas the parathyroids are not so regulated It seems unlikely that the parathyroid secretion would act on the cells of its origin

B Pregnancy

The mineral demands of the fetus do unquestionably place added strain on the mineral metabolism of the maternal organism Pregnancy itself, however, does not appear to introduce any complications in mineral metabolism other than drainage The maternal response is purely an adaptation to this depletion, as it would be to loss of mineral by any other route The average human infant contains 24 30 g of calcium at birth (28) Bone resorption very commonly occurs during pregnancy but it can be largely negated by maintenance of a positive calcium

balance Noting that in women the serum calcium level falls during pregnancy to the lower limits of the normal range (26,27,139), also that the parathyroids appear to be hyperplastic, and that the serum phosphatase is doubled or trebled in the last trimester, it is generally believed that a mild state of hyperparathyroidism exists The rather low level of calcium represents a temporary incomplete compensation on the part of the parathyroids Indeed, in pregnant rats demineralization of the skeleton may be seen with no diminution of serum calcium values (27) showing perhaps that the parathyroids have made complete compensation The evidence is particularly striking since removal of the parathyroids during pregnancy blocks the mobilization of mineral from the body depots (bone) and the blood calcium level falls (29) Bodansky and Duff (30) also found that fetal growth and storage of calcium and phosphorus is normal, in spite of large differences in maternal intake of calcium and phosphorus, only when the parathyroids are present, without them an abnormal mineral intake causes disturbances on both sides of the placenta

Sinclair's studies (187,188) on the increase in the volume of the parathyroids during pregnancy were based on the glands from rats used in the careful metabolism studies of Bodansky and Duff A simple hypertrophy of about 65% occurred during the first pregnancy on a mineral intake just adequate for reproduction, and the effect was cumulative with repeated pregnancies The extent of enlargement was almost doubled, and marked hyperplasia was produced by diets extremely deficient in calcium and low in phosphate Furthermore, the fetal parathyroid glands were depressed by high, and stimulated by low, maternal calcium levels The data of Opper and Thale (142) relating the effect of pregnancy to the volume of the parathyroids are in agreement with those of Sinclair

Two lines of evidence indicate quite clearly that a unidirectional passage exists with regard to the placental transmission of parathyroid hormone Carlson (37) found that fetuses did not protect the pregnant bitch against tetany following parathyroidectomy Parathyroidectomized rats likewise develop tetany two to four days before term, which is precisely the time that one might expect the greatest ameliorative effect from the fetal glands if their secretion was able to cross to the mother's blood stream (28) That the fetal glands are functional is definitely indicated by the fact that both in humans (27) and in dogs (107) the fetal serum calcium level is 1–2 mg % higher than that of the mother Fetal rat parathyroid glands are twice as large in proportion to body weight as those of adults, yet they constitute no more than 5% of the

total adult gland weight and on this basis alone could not be expected to afford much protection to the mother against total parathyroidectomy (188)

With respect to reproduction, it has been shown by Bodansky and Duff (28) that loss of the parathyroids greatly diminished fertility in rats and caused a reduction in the number and birth weight of fetuses The gestation period and labor were prolonged and the maternal and fetal mortality rates were high In Chandler's (41) extensive experience, prolongation of the gestation period was rarely seen Parathyroid changes in pseudopregnancy and after progesterone treatment have not received the attention they deserve The evidence at hand is very inconclusive (19,36)

C Lactation

Lactation can constitute a great drain on the mineral resources of the mother Mineralization of the skeleton in most animals, barely begun at birth, progresses rapidly during the period of nursing when the intake of mineral is entirely from the milk of the mother In parathyroidectomized rabbits copious lactation had a much greater tendency to bring out parathyroid deficiency symptoms than did pregnancy (53,55) Symptoms did not appear, however, if the litter was reduced to two Tetany was more severe in rats during lactation than during pregnancy, and this effect was more marked with large litters than with small ones (41,122)

It is not unusual to find a negative calcium balance during lactation, especially in dairy cows and women, and without special dietary precautions, skeletal reserves are attacked through the mediation of excess PTH production With adequate dietary calcium and vitamin D intake, this skeletal drain can be reduced to a minimum in humans and cattle, and apparently abolished in rats (36)

D Renal Insufficiency

The only experimental data on chronic renal insufficiency as concerned with partial nephrectomy Pappenheimer (144) removed one kidney in rats and greatly reduced the other so that the health of the animals was impaired, no chemical determinations were made on the blood or urine He obtained an increase in the volume of the parathyroids that varied with the severity of the kidney damage as determined on a pathological basis In no event was there more than an occasional suggestion of osteofibrotic change But, when the calcium intake was reduced, the bone lesions were greatly intensified and the parathyroid enlargement augmented Donohue *et al* (51) found that after partial nephrectomy

the calcium content of the remaining kidney tissue was increased and this could be prevented by removing the parathyroids

Selye (179) finds that demineralization of the skeleton which occurs after total nephrectomy in rats is completely prevented if the parathyroids are also removed As mentioned previously, a factor that must be considered in the bone destruction in these experiments is the accompanying acidosis Selye believes that the kidneys can only influence bone resorption through the parathyroids, and this is the exact opposite of the view which holds that the parathyroids can affect bone only if the kidneys are first able to excrete extra amounts of phosphate

XII. Alterations of Parathyroid Function in Man

A Hypoparathyroidism and Replacement Therapy

The principles of parathyroid physiology learned from a study of the effects of extirpation and of replacement therapy in animals, especially dogs, are almost entirely applicable to man Only brief mention of certain outstanding features which seem applicable to this discussion will be made as excellent recent reviews are available (3,150)

Hypoparathyroidism results either from accidental operative removal of the parathyroid glands or from secretory failure of unknown cause The operative cases develop severe symptoms within a day or so, but in idiopathic hypoparathyroidism the progress of the disease is often very slow and the early manifestations (numbness, tingling sensations, muscle cramps, etc) may not be particularly indicative of parathyroid involvement In addition to hypocalcemia, the well-known clinical signs of borderline hypoparathyroidism or latent tetany are Erb's (hypergalvanism), Chvostek's (muscular spasm from tapping facial nerve), and Trousseau's (spasm of muscles of forearm and hand when pressure is applied over the bicipital sulcus) True parathyroprival tetany commences with local and usually painful twitches or spasms The muscle involvement rapidly becomes more general and culminates in epileptiform convulsions and laryngeal stridor The blood serum calcium is invariably low and the inorganic phosphorus may reach 12 mg as compared to the normal level of about 3 5 mg % The urine is ordinarily calcium-free and the phosphate excretion may be normal to subnormal In long standing cases, the bones become more dense than normal, but the teeth which when not fully formed at the inception of the disease, show acalcification of the dentine during their subsequent development Evidence of enamel hypoplasia of the teeth appears not to be well authenticated Cataracts are often present and metastatic calcification of the brain has been seen, but this need not necessarily be associated with the diseased condition of the parathyroids (3,60)

Treatment is directed toward the relief of tetany This can be accomplished with dramatic suddenness by intravenous administration of calcium gluconate, but the effect is transient and other more sustaining measures must be used Calcium salts, particularly chloride, are given by mouth Parathyroid hormone is and can be a life-saving material, it requires approximately four hours to raise the serum calcium sufficiently to relieve tetany It has the disadvantage of producing painful injecting sites and may eventually become ineffective through the development of an immune reaction Calciferol, viosterol, and especially dihydrotachysterol, though slow in raising the serum calcium, are extraordinarily beneficial and widely used The patient is kept on an adequate calcium diet but milk is not indicated because of its high phosphate content If the blood calcium is kept a little low, the danger of kidney damage from excessive calcium excretion is avoided This adjustment is greatly aided by use of the Sulkowitch reagent,[1] which on merely being added to urine shows the relative abundance of calcium by the cloudiness of the white precipitate that forms

B Infantile Tetany

Infants sometimes exhibit tetany which is believed to be of hypoparathyroid origin This occurs most often during the first few days after birth It has been found that infants show a drop in the excretion of phosphate in 3–6 days and that the serum calcium is simultaneously low The hemorrhages which are often seen in the parathyroid glands of infants are not necessarily associated with the appearance of tetany Bakwin (16) from a study of the social distribution of formula feeding and infantile tetany, is of the opinion that cow's milk is the provocative agent It has about six times as much phosphate as breast milk There is no evidence that the tetany is due to a lack of vitamin D An important consideration in infantile tetany is posed by the case of a hypoparathyroid infant born to a mother with hyperparathyroidism (66) The infant developed tetany which was believed due to compensatory hypoplasia of the fetal glands It is well established that PTH injections induce parathyroid hypoplasia and there is reasonable certainty (see p 283) that the parathyroid hormone can pass from mother to fetus

C Primary Hyperparathyroidism

Primary hyperparathyroidism may derive from an adenoma of one or more of the parathyroid glands or from hypertrophy of all the glands The disease is characterized by depression of nerve irritability, stupor,

[1] Dissolve 2 5 g oxalic acid, 2 5 g ammonium oxalate, and 5 ml glacial acetic acid in distilled water to a volume of 150 ml

and coma Severe pain in joints and especially in the pelvis and lower limbs is frequently present The serum calcium is high and the plasma inorganic phosphate is below the patient's normal value but not markedly so There is excessive calcium elimination in the urine but not in the feces Rarefaction of bone (11) is very common but it is not a constant finding Hoffheinz (105) cites eighteen cases of parathyroid tumors showing no skeletal damage The extent of bone destruction is dependent upon the calcium balance If a positive calcium balance is maintained through dietary means, bone resorption need not supervene Surgical reduction of the abnormal parathyroid tissue to an amount that is estimated to be adequate to meet normal requirements for parathyroid secretion is the only means available for treating hyperparathyroidism (49) If the adenoma is confined to only one gland and it alone is removed, tetany may appear for a short time due to the fact that the remaining glands have been depressed, probably by the hypercalemia, and are unable to readjust promptly to an active secretory status

D Secondary Hyperparathyroidism and Renal-Parathyroid Interrelationships

There is no doubt that the kidneys and the parathyroids are intimately interrelated, a disturbance in the function of either very often produces an abnormality in the function or morphology, or both, of the other In a compilation of eighty-three cases of hyperparathyroidism, evidence of some type of renal damage was present in forty-three (4) There is also an abundance of evidence that inadequate renal function can create a demand for more than the normal production of parathyroid hormone In an attempt to compensate for this demand the parathyroids undergo enlargement The histological picture in such patients is usually described as a diffuse hyperplasia affecting all the glands alike When renal insufficiency is the causative factor in hyperparathyroidism, the chain of disturbed physiological processes is thought to be (a) phosphate retention, (b) reciprocal lowering of blood calcium level, (c) prolonged stimulation of the parathyroids, (d) parathyroid enlargement and hyperfunction with resulting bone destruction

Pappenheimer and Wilens (146) found that parathyroid enlargement may occur in any type of nephritis The weight of the combined parathyroid glands from seven cases of severe nephritis averaged 244 mg , in twenty-one cases of mild nephritis 177 mg , and in sixty-two normal adults 117 mg It is interesting that enlargement was also noted in a small group of cases that showed renal lesions but in which no symptoms of renal insufficiency were detected Highman and Hamilton (103) claim to have demonstrated PTH in the blood of patients suffering from

chronic renal disease by use of the Hamilton-Schwartz test (93) Gilligan *et al* (74) however, found this test to show a negative response in eighteen of nineteen trials on fifteen patients with severe renal insufficiency Until it has been determined that blood from bona fide cases of hyperparathyroidism will yield an unquestionably positive response, any data obtained by this technique can only be regarded with skepticism Albright (3) believes that in uncomplicated renal insufficiency the blood calcium does not fall in proportion to the rise in phosphate and this he attributes to the associated acidosis which favors calcium mobilization from bone and from the gut He states that, if the acidosis is controlled, tetany will appear This has all the earmarks of hypoparathyroidism and one might infer that compensatory parathyroid adjustment to the low calcium stimulation had not been made, but for his further comment that "it is probably the secondary hyperparathyroidism which prevents patients with renal insufficiency from having severe tetany" According to Ginzler and Jaffe (76) osseous demineralization in chronic renal insufficiency is due in most cases not to hyperfunction of the parathyroids but to acidosis They speculate that because of the impaired ability of the kidney to form base (ammonia) the body is forced to draw upon its stores of fixed base (Ca and Mg in bones) in order to eliminate the acid end products of metabolism Albright (3) recognizes a condition, called 'hyperhyperparathyroidism," or parathyroid poisoning, seen in dogs receiving massive doses of PTH and in rare clinical cases The unusual feature is that hypercalcemia and hyperphosphatemia coexist He believes that the high blood calcium results in renal blockage which leads to phosphate retention and hyperphosphatemia Herbert *et al* (101) described a patient with similar blood changes which they believe resulted initially from renal disease with phosphate retention and hypocalcemia followed by compensatory parathyroid hyperactivity

There is much that is speculative or circumstantial in the interpretation of the underlying causes of secondary hyperparathyroidism Most of the information available has been obtained from clinical material and often the only evidence that the renal disease preceded the parathyroid symptoms is based on case histories Furthermore the course of events has often been largely reconstructed from autopsy findings Duguid (57) has aptly phrased a note of caution in this connection "Parathyroid hyperplasia has frequently been observed, *postmortem*, in cases of chronic nephritis and it has been inferred therefrom that nephritis causes hyperparathyroidism The inference is by no means conclusive for there is equally good evidence that hyperparathyroidism causes nephritis and when the two conditions co-exist it is difficult to decide on morbid anatomical grounds which is primary" The extensive clinical data concerning chronic nephritis and parathyroid function do not rest on a

sound experimental basis there being to date not more than three papers in the latter category The principal reason for this situation is that no satisfactory method has been devised for producing a chronic nephritis in laboratory animals which will permit long survival and yield data pertinent to this problem There are many instances of alleged secondary hyperparathyroidism which are based on the finding of hyperplasia of the parathyroids with inconclusive evidence of hyperfunction This is especially true if we admit that the bone resorption in these cases may be due to acidosis The possibility that the parathyroid hyperplasia may be due to an inhibiting agent in cases where NPN and phosphate are being retained has not been seriously considered Attention can be properly directed to the hyperplasia with associated hypofunction of the thyroid when antithyroid compounds are given (12 130)

Goadby and Stacey (79) and Goadby (78) administered parathyroid hormone to normal and nephritic patients and found that the phosphate diuresis was inversely proportional to the renal insufficiency They laid emphasis on the fact that the plasma inorganic phosphate was increased only in the normal patients after PTH injection and this they maintain demonstrates that there is no mobilization of phosphate from skeletal reserves when the kidneys are nonfunctional The very significant fact that in every nephritic patient injected the 24 hour rise in serum calcium was as great as in normal persons must not be overlooked Goadby claims that PTH acts in at least two ways on the kidneys to promote phosphate excretion and on the mobilization of calcium from the skeleton Duguid (57) produced nephritis in rats using a diet of bread and potatoes with added phosphate and the parathyroids were always hypertrophic whether nephritis was present or not Chown *et al* (42 43) observed a chronic nephritis in rats which had been treated with parathyroid hormone for six months or more Obstructing calcium deposits were found inside and outside of the nephrons

Anderson (9) in a comprehensive review of kidney parathyroid relationships concludes that while hyperparathyroidism may produce renal failure it is nevertheless the underlying cause of only a very small percentage of renal calculi Be that as it may the formation of calcium phosphate or oxalate calculi is a common clinical feature of hyperparathyroidism and indeed Albright (2) finds the symptoms associated with nephrolithiasis one of the earliest manifestations of this disease

XIII Relation of the Parathyroids to Skeletal Growth, Bone Repair, and Dental Defects

No study of the effects of early removal of the parathyroids alone on skeletal growth appears to have been made Thyroparathyroidectomy in newborn rats has a pronounced stunting effect which is due almost

entirely to the removal of the thyroid, as was proven by replacement therapy with parathyroid extract alone and in combination with thyroid powder (167) Parathyroid ablation in young growing rats given a normal stock diet causes little or no interruption of growth Bodansky and Duff (30) find that the birth weight of fetuses of thyroidectomized mothers is smaller than those from normal mothers

Silberberg and Silberberg (185,186) have made a most careful histological study of the effect of PTH on the skeletal changes in normal growing mice on adequate calcium intake Notably, PTH causes hypertrophy, calcification and disintegration of the epiphyseal disc in growing animals without causing proliferation and stimulates osseous formation A resorptive process then sets in which tends to close the epiphyses, and in this manner simulates and accelerates skeletal aging In old mice, increased bone formation and increased calcification of inactive epiphyseal cartilage occurs The changes induced by administering calcium gluconate differ from the above, mainly in that the resorptive phase does not take place When PTH was combined with calcium gluconate, the aging effect was not intensified over that produced with PTH alone, but bone formation was accelerated in either growing or adult mice

There is a considerable species difference in the response of skeletal tissue to PTH injections Definite stunting has been observed in rats (17,33,178) and puppies (112) whereas no definite alteration of bone growth was seen in cats (17) Imperfect ossification and bowing of bones has been recorded in dogs and rats (Fig 5) The discrepancies are in part attributable to the difference in the ages of the animals studied and to their diet, but mainly to the difference in amount of hormonal stimulation and length of treatment In rats, initial demineralization is usually succeeded by hyperossification during prolonged parathyroid treatment (179) The early stage of osteoclastic proliferation and bone salt removal is followed by fibrous invasion and bone destruction (including the matrix) leading to the condition known as "osteitis fibrosa" At this time, a proliferation of osteoblasts leads to formation of hypercalcified "marble" bone Bone abnormalities are a common but not a necessary accompaniment of hyperparathyroidism in man Albright has pointed out that the bone disorders depend upon a negative mineral balance It is not settled whether the decline in osteitis fibrosa in experimental animals is associated with the development of an immunity to the hormone The fact that marble bone disease does not develop in the hyperparathyroidism of man may be related to the failure to develop immunity to endogenous hormone

The bone trabeculae are favored sites for release of mineral under PTH influence When resorption of the cortex does occur, it is most

pronounced subjacent to the epiphyses With continued treatment the major feature of the bones, split lengthwise and examined grossly, is increased compactness of the trabeculae at the ends of the shafts, eventually even this process is replaced by loss of trabeculae and a return to nearly normal bone structure Resorption of the cortex with tendency to spontaneous fracture must be placed in the category of extreme parathyroid overdosage (for details see 12 33,112,116 117,178,179)

Membrane bones react in the same way as endochondral bones The diploe of the skull bones becomes denser but the shape of the skull is not altered in rats stunted by PTH (33)

The repair of fractures during states of greatly altered parathyroid function has attracted considerable study because of the possibility of *influencing the healing process* *It has been repeatedly shown that the* deposition of mineral in the callus is delayed by parathyroidectomy (52,180), but nevertheless bone repair goes on to completion The observations of the early workers were far more striking in this connection, probably due, as Shelling points out, to the poor dietary conditions employed High calcium or high vitamin D intakes, separately or in combination, have a distinctly beneficial effect in rats on the calcific mending, but *their maximum effect is improved by (or exceeded alone by)* parathyroid extract (180) Parathyroid hormone increased the amount of the mineral deposited in calluses in pigeons (162) even in the presence of some demineralization of the skeleton This is not unexpected, as bone deposition and bone resorption are not mutually exclusive processes The formation of the callus *per se* appears not to be influenced by the parathyroids (For a discussion of the many systemic factors concerned in bone mending see Armstrong 11 No agent has yet been found which is of any clinical value in this connection)

The effect of chronic estrogen treatment on the skeletal system of the mouse has been studied in great detail by Gardner (69) It is noteworthy that the excessive osseous formation in the marrow cavity of the long bones and the resulting increase in their breaking strength (femurs) were as readily attained with estrogen in parathyroidectomized mice as *in intact animals (personal communication)*

The striking acalcification of the dental matrix and dental fractures noted by Erdheim (63), and others, in parathyroidectomized rats again appears to have been grossly exaggerated by the probable rachitogenic property of the diet Certainly the dental disturbances seen by Schour *et al* (174) in parathyroidectomized rats are a very different matter They found that during the first twenty days after operation the dentine was actually hypercalcified and subsequently there appeared alternating zones of hypo- and hypercalcification There was, however, a general

tendency toward poor calcification and the incremental pattern showed a wavy stratification with vascular tufts extending into the dentine In animals surviving many months, and especially after repeated pregnancy and lactation, the incisors did fracture The slowing of the rate of eruption of the incisors noted by others was not observed by Schour *et al* (174) in properly controlled experiments A single injection of PTH into a normal rat led to the deposition of a hypocalcified stripe in the dentine followed by a hypercalcified one (175)

Idiopathic hypoparathyroidism in children results in the apposition of uncalcified matrix and as a consequence the softened apices of the roots become traumatically blunted and bent Parathyroid hormone will correct only that portion of the tooth which is laid down following substitution therapy

In any event calcium is never removed from either the dentine or the enamel and one should not speak of a poorly calcified dental structure as decalcified, they are, strictly, acalcified Attention is properly directed by Strock (193) to the frequency of oral symptoms in cases of hyperparathyroidism Osteoporosis of the mandible, cyst formation epulis, and absence of the lamina dura, though not of specific diagnostic value, should be given the consideration that the present knowledge of parathyroid physiology warrants

XIII. Mineral Appetite

The self-selection experiments of Richter and co-workers have shown very clearly that rats with a dietary deficiency are able to choose substances needed to restore health The mineral appetite of rats (155–158) with altered parathyroid function has reflected in a strikingly clear manner the mineral demands of the organism under these conditions Intact adult rats on a low calcium diet and offered a choice of distilled water or a solution of calcium lactate consume about 20 ml of water and 1–2 ml of the calcium solution daily After parathyroid ablation, the calcium lactate consumption jumps to over 20 ml per day, the water intake falls to approximately 4 ml (Fig 7), and tetany is uniformly averted Blood calcium studies were not made but it can be assumed that the increased calcium intake would help maintain the blood levels The injection of PTH in the operated rats reduced their calcium consumption but the amount which was required to bring it to the preoperative level was toxic Vitamin D and AT 10 (dihydrotachysterol) were also effective in reducing the calcium intake to normal After mixing calcium lactate with the food, the consumption of calcium lactate in the drinking water gradually declined Parathyroidectomized animals also have an increased appetite for strontium and magnesium Recalling that para-

thyroidectomized animals exhibit phosphatemia, Richter was interested to inquire whether they might not also refuse something of which they have an overabundance, namely phosphorus Normal rats on a low phosphorus diet and given a choice of 1% dibasic sodium phosphate or water readily gave the answer by refusing phosphate after they had been parathyroidectomized Injections of parathyroid extract or feeding AT-10 restored the phosphorus intake to normal These experiments

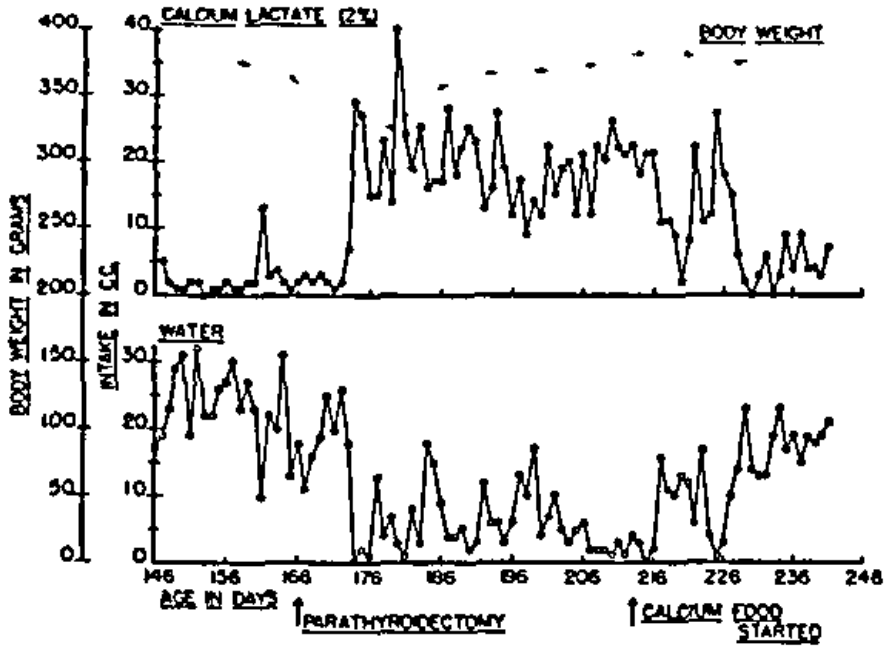


FIG 7 —Indicating the calcium and water intake in rats before and after removal of the parathyroid glands (From Richter and Birmingham 155)

also revealed an interesting fact concerning anorexia in parathyroidec tomized animals By alternately offering operated rats a choice of taking phosphate in their food or drinking water, it was established that the parathyroidectomized rat had no anorexia and that he refused food only to avoid phosphate consumption

Using a similar technique, Wilens and Waller (213) studied the cal cium and phosphorus appetite of rats after partial nephrectomy They found that rats on a low-calcium diet with a choice of distilled water or calcium lactate met their increased thirst after nephrectomy by consuming water Partial nephrectomy did not result in a reduced consumption of phosphate as would be anticipated, but the experiments were not conclusive since the blood phosphate level was not determined

ADDENDUM

Since this manuscript was prepared significant progress has been made in the assay, preparation, and purification of parathyroid hormone

Tepperman *et al* (197a) found that the subcutaneous injection of parathyroid hormone into adult rats resulted in a fall in the serum inorganic phosphorus which was directly proportional to the logarithm of the dose of hormone administered (in *U S P* units) This assay appears to have decided advantages in the estimation of parathyroid activity demanded for work on the isolation of this hormone L'Heureux *et al* (102a) devised a method for preparing a fatfree gland powder which is especially suitable starting material for extraction procedures Using 0 2 *N* hydrochloric acid at not more than 70–80°C they obtained an extract with high total activity The neutralized acid extract fractionated with 86% neutral acetone or 90% acid acetone yielded a 7–10 fold increase in activity per unit nitrogen Their preparation is comparable to that of Ross and Wood in order of nitrogen potency, protein character, solubility, and freedom from a carbohydrate moiety On ultracentrifugation, however, their preparation appeared not to contain the high molecular weight protein noted by Ross and Wood The electrophoretic analysis showed evidence of at least two components

A study of the distribution, retention and excretion of radioactive phosphorus during states of hypo and hyperthyroidism in rats (Tweedy *et al*, 206a) has provided additional evidence for the belief that para thyroid hormone acts directly upon the kidney During the first 24 hours following thyroparathyroidectomy a larger percentage of an injected dose of labeled phosphorus was retained by the various tissues of the body than occurred in similarly injected control animals This was a conse quence of delayed excretion The ability of such operated rats to excrete phosphorus gradually improved as the postoperative interval lengthened and this reached normal values at 15 to 25 days The injections of para thyroid hormone soon after the operation brought about a prompt urinary excretion of labeled phosphorus and resulted in normal retention pattern in the tissue of the unexcreted portion After bilateral nephrectomy, the injection of parathyroid hormone had no influence on the distribution retention or excretion of radiophosphorus

REFERENCES

1 Abel J J Geiling E M K Rouiller C A Bell F K, and Wintersteiner, O *J Pharmacol* **31** 65 85 (1927)
2 Albright F *New Engl J Med* **209** 476–480 (1941)
3 Albright F Glandular phys ology and therapy Chap 26 Am Med Assoc 1942
4 Albright F Baird P C Cope O and Bloomberg E *Am J Med Sci* **187** 49–65 (1934)
5 Albr ght F Bauer W Ropes M and Aub J C *J Clin Invest* **7**, 139–181 (1929)
6 Albright F and Ellsworth R *ibid* **7**, 183 201 (1929)

7 Albright, F , Sulkowitch, H W , and Bloomberg, E *ibid* **18**, 165–169 (1939)
8 Allardyce, W J *Am J Physiol* **98**, 417–429 (1931)
9 Anderson, W A D *Arch Path* **27**, 753–778 (1939)
10 Anselmino, K J , Hoffman, F , and Herold, L *Klin Wochschr* **13**, 45–47 (1934)
11 Anspach, W E , and Clifton, W M *Am J Diseases Children* **58**, 540–557 (1939)
12 Astwood, E. B , Sullivan, J , Bissell, A , and Tyslowitz, R. *Endocrinology* **32**, 210–225 (1943)
13 Aub, J C , Bauer, W , Heath, C , and Ropes, M *J Clin Invest* **7**, 97–137 (1929)
14 Babkin, B P , Komarov, O , and Komarov, S A *Endocrinology* **26**, 703–715 (1940)
15 Baker, B L. *Anat Record* **83**, 47–73 (1942)
16 Bakwin, Harry *J Pediat* **14**, 1–10 (1940)
17 Bauer, W , Aub, J C , and Albright, F *J Exptl Med* **49**, 145–161 (1929)
18 Baumann, E J , and Sprinson, D B *Am J Physiol* **125**, 741–746 (1939)
19 Bastenie, P , and Zylberzac, S *Compt rend soc biol* **132**, 93–94 (1939)
20 Bedinger, P L , Kendrick, A B , and Keeton, R W *J Pharmacol* **74**, 1–10 (1942)
21 Berman, L. *Proc. Soc Exptl Biol Med* **21**, 465 (1924)
22 Bliss, C I , and Rose, C L *Am J Hyg* **31**, 79–98 (1939)
23 Blumenfeld, C M , and Rice, H M *Anat Record* **70**, 227–233 (1938)
24 Blumenthal, H T , and Loeb, L *Endocrinology* **30**, 502 510 (1942)
25 Blumenthal, H T , and Loeb, L *ibid* **30**, 502–510 (1942)
26 Bodansky, M *Am J Clin Path* **9**, 36–51 (1939)
27 Bodansky, M , and Duff, V B *J Am Med Assoc* **112**, 223–229 (1939)
28 Bodansky, M , and Duff, V B *J Nutrition* **21**, 179–192 (1941)
29 Bodansky, M , and Duff, V B *ibid* **21**, 235–242 (1941)
30 Bodansky, M , and Duff, V B *ibid* **22**, 25–41 (1941)
31 Brink, F , and Bronk, D W *Proc Soc Exptl Biol Med* **37**, 94–95 (1937)
32 Brull, L., and Carbonesco, G *Compt rend soc biol* **131**, 800–801 (1939)
33 Burrow, R B *Am J Anat* **62**, 237–290 (1938)
34 Cantarow, A , Haurey, V G , and Whitbeck, C G *Proc Soc Exptl Biol Med* **39**, 15–17 (1938)
35 Cantarow, A , Stewart, H L , and Housel, E L. *Endocrinology* **22**, 13 27 (1938)
36 Campbell, I L , and Turner, C W *Agr Expt Sta Missouri Res Bull* **352** Columbia, Missouri, p 134, 1942
37 Carlson, A J *Proc. Soc Exptl Biol Med* **10**, 183–184 (1913)
38 Carlson, A J , and Jacobson, C *Am J Physiol* **28**, 133–160 (1911)
39 Carnes, W H , Osbold, J , and Stoerk, H C *ibid* **139**, 188–192 (1943)
40 Carnes, W H , Pappenheimer, A M , and Stoerk, H C *Proc Soc Exptl Biol Med* **51**, 314–316 (1942)
41 Chandler, S B *Anat Record* **53**, 105–120 (1932)
42 Chown, B , Lee, M , and Teal, J *Can Med Assoc J* **36**, 7–10 (1937)
43 Chown, B , Lee, M , Teal, J , and Currie, R *J Path Bact* **49**, 273–290 (1939)
44 Collip, J B *J Biol Chem* **63**, 396–438 (1925)
45 Collip, J B *Harvey Lectures* 113–172 (1925–1926)
46 Collip, J B , and Clark E P *J Biol Chem* **66**, 133–137 (1925)
47 Collip, J B , Clark, E P , and Scott, J W *ibid* 439–460 (1925)
48 Collip, J B , Pugsley, L I Selye H , and Thomson, D I *Brit J Exptl Path* **15**, 335–336 (1934)

49 Cope, O *Ann Surg* **114**, 706–733 (1941)
50 De Robertis, E *Anat Record* **79**, 417–433 (1941).
51 Donohue, W, Spingarn, C, and Pappenheimer, A M *J Exptl Med* **66**, 697–704 (1937)
52 Dragstedt, C A *Am J Physiol* **97**, 517 (1931)
53 Dragstedt, L R *Physiol Revs* **7**, 499–530 (1927)
54 Dragstedt, L R Quoted by Patt and Luckhardt as a personal communication, 1942
55 Dragstedt, L R, Sudan, A C, and Phillips, K *Am J Physiol* **69**, 477–497 (1924)
56 Drake, T G, Albright, F, and Castleman, B *J Clin Invest* **16**, 203–206 (1937)
57 Duguid, J B *J Path Bact* **54**, 177–181 (1942)
58 Durlackber, S H, and Darrow, D C *Am J Physiol* **136**, 577–583 (1942)
59 Dyer, F J *J Physiol* **75**, 13P (1932)
60 Eaton, L M, and Haines, S F *Proc Staff Meetings Mayo Clinic* **14**, 48 (1939)
61 Ellsworth, R *J Clin Invest* **11**, 1011–1017 (1932)
62 Ellsworth, R, and Futcher, P H *Bull Johns Hopkins Hosp* **57**, 91–98 (1935)
63 Erdheim, J *Denkschr math naturw Klasse Kaiserl Akad Wissensch Wien* **90**, 363–683 (1914)
63a Esau, J N, and Stoland, O O *Am J Physiol* **92**, 25–34 (1930)
64 Fay, M, Behrmann, V G, and Buck, D M *ibid* **136**, 716–719 (1942)
65 Fisher, N F, and Larson, E *Proc Soc Exptl Biol Med* **22**, 447–448 (1925)
66 Friderischsen, C *Lancet* **1**, 85–86 (1939)
67 Friedgood, H B *Endocrinology* **20**, 159–170 (1936)
68 Friedgood, H B, and McLean, R *Am J Physiol* **118**, 588–593 (1937)
69 Gardner, W U *Endocrinology* **32**, 149–160 (1943)
70 Gellhorn, E *Am J Physiol* **111**, 466–476 (1935)
71 Gersh, I *Anat Record* **70**, 331–350 (1938)
72 Gersh, I *Am J Physiol* **12**, 589–594 (1938)
73 Gilligan, D R, Volk, M C, and Altschule, M D *J Biol. Chem* **103**, 745–756 (1933)
74 Gilligan, D R, Volk, M C, and Gargill, S L *J Clin Invest* **17**, 641–647 (1938)
75 Gilmour, J R, and Martin, W J *J Path Bact* **44**, 431–462 (1937)
76 Ginzler, A M, and Jaffe, H L *Am J Path* **17**, 293–302 (1941)
77 Gley, E *Compt rend soc biol* **43**, 841–847 (1891)
78 Goadby, J K *Biochem J* **31**, 1530–1533 (1937)
79 Goadby, J K, and Stacey, R. S *ibid* **30**, 269–272 (1936)
80 Godwin, M C *Anat Record* **68**, 305–326 (1937)
81 Grafflin, A L *Endocrinology* **26**, 857–859 (1940)
82 Grafflin, A L *ibid* **30**, 571–573 (1942)
83 Greenberg, D M, and Gunther, L *Arch Internal Med* **50**, 855–875 (1932)
84 Greenwald, I *Am J Physiol* **28**, 103–132 (1911)
85 Greenwald, I *J Biol Chem* **61**, 649–651 (1924)
86 Greenwald, I *ibid* **67**, 1–28 (1926)
87 Greenwald, I, and Gross, J *ibid* **66**, 185–199 (1925)
88 Greenwald, I, and Gross, J *ibid* **66**, 217–227 (1925)
89 Greep, R O Unpublished, 1946
90 Hadfield, G, and Rogers, H *J Path Bact* **35**, 259–263 (1932)
91 Ham, A W, and Haist, R E *Nature* **144**, 835 (1939)
92 Ham, A. W, Littner, N, Drake, T G, Robertson, E C, and Tisdall, F F *Am J Path* **16**, 277–286 (1940)

93 Hamilton, B, and Schwartz, C *J Pharmacol* **46**, 285–292 (1932)
94 Hanson, A M *Proc Soc Exptl Biol Med* **22**, 560–561 (1925)
95 Hanson, A M *J Am Med Assoc* **90**, 747–748 (1928)
96 Hastings, A B, and Murray, H A *J Biol Chem* **46**, 233–256 (1921)
97 Harrison, H E, and Harrison, H C, *Am J Physiol* **134**, 781–788 (1941)
98 Harrison, H E, and Harrison, H C *J Clin Invest* **20**, 47–55 (1941)
99 Heinback, W F, Jr *Anat Record* **57**, 251–262 (1933)
100 Helfet, A J *Brit J Surg* **27**, 651–677 (1940)
101 Herbert, F K, Miller, H G, and Richardson, G O *J Path Bact* **53**, 161–182 (1941)
102 Hertz, S, and Kranes, A *Endocrinology* **18**, 350–360 (1934)
102a L'Heureux, M V, Tepperman H M, and Wilhelmi, A E *J Biol Chem* **168**, 167–176 (1947)
103 Highman, W J, Jr, and Hamilton, B *J Clin Invest* **16**, 103–105 (1937)
104 Hjort, A M, Robinson, S C, and Tendick, F H *J Biol Chem* **65**, 117–128 (1925)
105 Hoffheinz *Arch Path Anat Physiol Virchow's* **256**, 705–735 (1925)
106 Hoskins, M M, and Chandler, S B *Anat Record* **30**, 95–98 (1925)
107 Hoskins, F M, and Snyder, F F *Am J Physiol* **104**, 530–536 (1933)
108 Houssay, B A, and Sammartino, R *Compt rend soc biol* **114**, 729–732 (1933)
109 Hueper, W *Arch Path* **3**, 14–25 (1927)
110 Ingalls, T H, Donaldson, G A, and Albright, F *J Clin Invest* **22**, 603–608 (1943)
111 Jackson, C M *Am J Anat* **19**, 305–352 (1916)
112 Jaffe, H L, and Bodansky, A *J Exptl Med* **52**, 669–694 (1930)
113 Jaffe, H L, Bodansky, A, and Blair, J E *Arch Path* **11**, 207–228 (1931)
114 Jaffe, H L, Bodansky, A, and Blair, J E *J Exptl Med* **55**, 139–154 (1932)
115 Jensen, J, and Evans, E A *Physiol Revs* **14**, 188–209 (1936)
116 Johnson, J L. *Am J Med Sci* **183**, 761–769 (1932)
117 Johnson, J L *ibid* **183**, 769–775 (1932)
118 Jones, J H *J Biol Chem* **115**, 371–379 (1936)
119 Juhn, M, and Mitchell, J B *Am J Physiol* **88**, 177–182 (1929)
120 Kim, M H *Acta Med Keijo* **12**, 203 (1929) Cited by Selye, 1942 (see 179)
121 Kozelka, F L. *Am J Physiol, Suppl* **93**, (1936)
122 Kozelka, F L, Hart, E B, and Bohsted, G *J Biol Chem* **100**, 715–729 (1933)
123 Learner, N, and Brown, C L *J Clin Endocrinol* **3**, 261–265 (1943)
124 Lederer, L G, and Crandall, L A *Am J Physiol* **118**, 52–56 (1931)
125 Loeb, J *ibid* **3**, 383–396 (1899–1900)
126 Logan, M A *J Biol Chem* **127**, 711–719 (1939)
127 Logan, M A, Christenson, W P, and Kirklin J W *Am J Physiol* **135**, 419–425 (1942)
128 MacCallum, W G, and Voegtlin, C *Bull Johns Hopkins Hosp* **19**, 91–92 (1908)
129 MacCallum, W G, and Voegtlin, C *J Exptl Med* **11**, 118–151 (1909)
130 MacKenzie, C G, and MacKenzie, J B *Endocrinology* **32**, 185–209 (1943)
131 Marine, D *Proc Soc Exptl Biol Med* **11**, 117–118 (1914)
132 Marlow, H W, and Koch, F C *Endocrinology* **21**, 72 84 (1937)
133 Maxwell, J P *Proc Roy Soc Med* **23**, 639–652 (1930)
134 McJunkin, F A, Tweedy, W R, and McNamara, E W *Am. J Path* **13**, 325–335 (1937)
135 McLean, F C, and Bloom, W *Science* **85**, 24 (1937)

136 McLean, F C, and Bloom, W *Arch Path* **32**, 315–33 (1941)
137 McLean, F C, and Hastings, A B *J Biol Chem* **108**, 285–322 (1935)
138 Monahan, E P, and Freeman, S *Am J Physiol* **142**, 104–106 (1944)
139 Mull, J W, and Bill, A H *Am J Obstet Gynecol* **23**, 807–814 (1932)
140 Nathanson, I T, Brues, A M, and Rawson, R W *Proc Exptl Biol Med* **43**, 737–740 (1940)
141 Neufeld, A H, and Collip, J B *Endocrinology* **30**, 135–141 (1942)
142 Opper, L, and Thale, T *Am J Physiol* **139**, 406–409 (1943)
143 Overholser, M D *Anat Record* **41**, 303–321 (1929)
144 Pappenheimer, A M *J Exptl Med* **64**, 965–980 (1936)
145 Pappenheimer, A M, and Johnson, J W *Proc Soc Exptl Biol Med* **38**, 777–779 (1938)
146 Pappenheimer, A M, and Wilens, S L *Am J Path* **11**, 73–91 (1935)
147 Patt, H M, and Luckhardt, A B *Endocrinology* **31**, 384–392 (1942)
148 Pfeiffer, C, Roby, C C, Dreisbach, R, and Glass, H G *ibid* **27**, 818–824 (1940)
149 Pierre, M, de Boissezon, P, and Lombard, C *Compt rend soc biol* **130**, 341–342 (1939)
150 Pope, A, and Aub, J C *New Engl J Med* **230**, 698–707 (1944)
151 Pugsley, L I *J Physiol* **76**, 315–328 (1932)
152 Pugsley, L I, and Collip, J B *Am J Physiol* **113**, P108 (1935)
153 Pugsley, L. I, and Selye, H *J Physiol* **79**, 113–117 (1933)
154 Reed, C I, Lackey, R W, and Payte, J I *Am J Physiol* **86**, 176–188 (1928)
155 Richter, C P, and Birmingham, J R. *Endocrinology* **29**, 655–666 (1941)
156 Richter, C P, and Eckert, J F *ibid* **21**, 50–54 (1937)
157 Richter, C P, and Eckert, J F *Am J Med Sci* **198**, 9–16 (1939)
158 Richter, C P, and Helfrick, S *Endocrinology* **33**, 349–352 (1943)
159 Riddle, O, and McDonald, M P *ibid* **36**, 48–52 (1945)
160 Riddle, O, Rouch, V M, and Smith G C *ibid* **36**, 41–47 (1945)
161 Roby, C C, Smith, S, and Pfeiffer, C *Am J Physiol* **129**, 766–772 (1940)
162 Roche, J, and Mourgue, M *Compt rend soc biol* **130**, 1138–1141 (1939)
163 Rose, W B, Stucky, C J, and Cowgill, G R *Am J Physiol* **91**, 554–562 (1930)
164 Rosenblueth, A, and Cannon, W B *ibid* **130**, 205–229 (1940)
165 Rosof, J A *J Exptl Zool* **68**, 121–157 (1934)
166 Ross, W F, and Wood, T R *J Biol Chem* **146**, 49–58 (1942)
167 Salmon, T N *Endocrinology* **23**, 446–457 (1938)
168 Salvesen, H A *J Biol Chem* **56**, 443–456 (1923)
169 Salvesen, H A, and Linder, G C *ibid* **58**, 635–639 (1924)
170 Sandstrom, I *Upsala Lakarforen. Forhand* **15**, 444–471 (1879–80) Translated by Seipel, C M, The Johns Hopkins Press, Baltimore
171 Saxton, J A, and Ellis, G M *Am J Path* **17**, 590 (1941)
172 Schiffrin, M J *Am J Physiol* **135**, 660–669 (1942)
173 Schmidt, C L A, and Greenberg, D M *Physiol Revs* **15**, 297–434 (1935)
174 Schour, I, Chandler, S B, and Tweedy, W R *Am J Path* **13**, 945–970 (1937)
175 Schour, I, Tweedy, W R, and McJunkin, F A *ibid* **10**, 321–342 (1934)
176 Schrire, V *S African J Med Soc* **6**, 1–5 (1941)
177 Schryver, S B, and Thumann, K *Biochem J* **21**, 1284–1301 (1927)
178 Selye, H *Endocrinology* **16**, 547–558 (1932)
179 Selye, H *Arch Path* **34**, 625–632 (1942)
180 Shelling, D H *Proc Soc Exptl Biol Med* **28**, 306–307 (1930)

181 Shelling, D H *J Biol Chem* 96, 195–214 (1932)
182 Shelling, D H The Parathyroids in Health and Disease C V Mosby, St Louis, Missouri, 1935
183 Shelling, D H, and Ascher, D E *J Biol Chem* 96, 195–214 (1932)
184. Shelling, D H, Kajdi, L., and Guth, L *Endocrinology* 22, 225–235 (1938)
185 Silberberg, M, and Silberberg R. *Am J Path* 19, 839–859 (1943)
186 Silberberg, M, and Silberberg, R. *Arch Path.* 36, 512–531 (1943)
187 Sinclair, J G *Anat Record* 80, 479–496 (1941)
188 Sinclair, J G *J Nutrition* 23, 141–152 (1942)
189 Smith, P E *Am J Anat* 45, 205–273 (1930)
190 Snyder, F F., and Tweedy, W R *Proc Soc Exptl Biol Med* 47, 234–236 (1941)
191 Steck, I E., Miller, D S, and Reed, C I *Am J Physiol* 110, 1–3 (1934)
192 Stewart, C P, and Percival, G H *Biochem J* 21, 301–313 (1927)
193 Strock, M S *New Engl J Med* 224, 1019–1023 (1941)
194 Stoerk, H C *Proc Soc Exptl Biol Med* 54, 50–53 (1943)
195 Stoerk, H C, and Carnes, W H *J Nutrition* 29, 43–50 (1945)
196 Swingle, W W, and Nicholas, J S *Am J Anat* 36, 91 129 (1925)
197 Taylor, N B, Weld, C B, Branion, H D, and Kay, H D *Can Med Assoc. J* 24, 763–777, 25, 20–35 (1931)
197a Tepperman H M L Heureux, M V and Wilhelmi A E *J Biol Chem* 168, 151–165 (1947)
198. Thomson, D L., and Collip, J B *Physiol Revs* 12, 309–383 (1932)
199 Tibbetts, D M, and Aub, J C *J Clin Invest* 16, 503–509 (1937)
200 Tobin, C E *Am J Anat* 65, 151–177 (1939)
201 Truszkowski, R, Blauth-Opiénska, J, and Iwanowska, J *Biochem J* 33, 1005–1911 (1939)
202 Tweedy, W R *ibid* 88, 649–657 (1930)
203 Tweedy, W R, Bell W P, and Vicens-Rios, C *ibid* 108, 105–112 (1935)
204 Tweedy, W R, and Campbell, W W *ibid* 154, 339–347 (1944)
205 Tweedy, W R and Chandler S B *Am J Physiol* 88, 754 760 (1929)
206 Tweedy, W R Chilcote M E, and Patras, M C *Federation Proc* 5, 159 (1946)
206a Tweedy W R Chilcote M E, and Patras M C *J Biol Chem* 168, 597 611 (1947)
207 Tweedy, W R, Smullen, G H, and Bell, W P *J Biol Chem* 116, 163–167 (1936)
208 Tweedy, W R., Templeton R D, and McJunkin F A *Am J Physiol* 115, 514–519 (1936)
209 Tweedy, W R, Templeton, R. D, and McJunkin, F A *Endocrinology* 21, 55–59 (1937)
210 Tweedy, W R, Templeton, R D, Patras, M C, McJunkin F A and McNamara, E W *J Biol Chem* 128, 407–415 (1939)
211 Tweedy, W R, and Torigne, M *ibid* 155–164 (1932)
212 Valenzuela, F, Huidobro, F, and Valdés R *Proc Soc Exptl Biol Med* 57, 31–33 (1944)
213 Wilens, S L., and Waller, R K *Endocrinology* 28, 828 834 (1941)
214. Wilson, D W, Stearns, T and Janney, J H *J Biol Chem* 21, 169–177 (1915)
215 Winkler, A W, Smith, P K, and Hebbel, E H *J Clin Invest* 21, 419–421 (1942)
216. Wood, T R., and Ross, W F *J Biol Chem* 146, 59–62 (1942)

Chapter VIII

The Internal Secretion of the Pancreas[1]

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I Introduction

With the exception of the liver, the pancreas is the largest gland connected with the alimentary tract It is a pink white organ which lies in

[1] This article does not purport to be a complete review of all existing information on the subject For detailed description and bibliography concerning the earlier investigations the reader is referred to various reviews (52 55 76 80 88 91 157 173 181)

the retroperitoneum at about the level of the second and third lumbar vertebrae In the adult it measures from 20 to 25 cm in length and varies in weight from 65 to 160 g Its right extremity, the "head," is the larger and is directed downward, the left extremity, or "tail," is transverse and terminates close to the spleen The pancreas consists of an exocrine portion, which elaborates certain digestive enzymes, and an endocrine portion, whose internal secretion plays an important part in the control of the carbohydrate metabolism of the body The exocrine and endocrine functions of the pancreas are carried out by distinctly different groups of cells

The present review is concerned only with the endocrine function of the pancreas The importance of this endocrine function becomes apparent upon examination of the physiological disturbances in the body which may be observed in total pancreatectomy or diabetes mellitus The following symptoms have been found to be characteristic

(1) Pronounced hyperglycemia and glycosuria
(2) Depletion of the glycogen stores in certain tissues (liver, muscle)
(3) Lowering of the respiratory quotient
(4) Increase in the NPN excretion
(5) Increased formation of ketones (ketosis)

II. History[2]

The first observations on the effects of the removal of the pancreas from animals were made at a time when a relationship between the function of the pancreas and diabetes was unsuspected As early as 1682, von Brunner (22) and, several years later, Haller (19, cited from Bouchardat) removed the pancreas from dogs, but they could observe no ill effects, the animals continuing to live apparently in good health Bérard and Colin (11) also extirpated the pancreas from dogs and likewise were unable to note any unfavorable effects after the operation Klebs and Munk (102) in 1869 seem to have been the first to undertake such extirpation for the purpose of demonstrating a possible relation of the pancreas to diabetes Their results, however, were also negative The failure of these earlier experimenters to demonstrate the essential nature of the pancreas was due, without doubt to incomplete removal of the organ Experiments of a different type were performed by Claude Bernard (12) in 1856 and later by Schiff (154), in 1872, who observed that blocking the pancreatic ducts with paraffin did not affect the health of the animals

In 1890 two clinicians, von Mehring and Minkowski (122), discovered that the complete removal of the pancreas from dogs was followed by symptoms which closely resembled those observed in human diabetes mellitus They deduced that these diabetic symptoms were evoked by

[2] See also the comparative history of different hormones in Chapter I

the lack of some specific function of the pancreas At that time clinical evidence already indicated that diabetes mellitus was associated in some way with the pancreas The experiments of von Mehring and Minkowski were the first decisive demonstration of such a relationship At the same time, and independently de Dominicis (42) made a similar observation Lépine in 1893 repeated these experiments and confirmed these findings (110) He advanced the theory that the pancreas elaborates an internal secretion which controls carbohydrate metabolism Proof of this was furnished by transplantation experiments performed in 1892 by both Minkowski (127,128) and Hédon (74,75) Portions of the pancreas were removed, grafted under the skin of dogs, and allowed to remain there until the circulation had been re-established, the rest of the gland was then removed In this way diabetic symptoms could be prevented, or at least greatly delayed On removal of the graft, the typical symptoms of pancreatectomy immediately appeared Gley (60 61) observed that tying the pancreatic veins thereby stopping the supply of blood from the pancreas, was followed by diabetic symptoms All these results could obviously be explained on the basis of the theory of an internal pancreatic secretion It was already known at that time that the pancreas formed an enzyme secretion necessary for digestion It was hardly to be expected, however, that the principle regulating carbohydrate metabolism was produced by the same tissue of the pancreas that was responsible for the elaboration of the digestive enzymes

III Islets of Langerhans

In 1869 Langerhans (108) described the presence in the pancreas of an epithelial tissue different from the alveoli and the ducts which convey the external secretion (enzymes) to the duodenum Langerhans described these cells but had no knowledge of their actual function

These "islets of Langerhans" originate from the pancreatic ducts, as do the alveoli The islets are structures distinct and apart from the rest of the pancreas and can easily be differentiated from the acinar tissue The islet tissue is present in abundance in the pancreas of most animals In normal human adults the pancreas contains 0 9%–2 7% of islet tissue, while the majority of diabetic people have less than 0 9% On the other hand, children may possess as much as 3 6% (171) It has been found that there are more islets in the tail (splenic end) than in the body or head of the pancreas The rich blood supply of the islets is an indication of their physiological significance The islets have been found to vary widely in size and number, even within a single species The total number of the islets in the human pancreas lies between 250,000 and 2,500,000, the majority of cases approximating 500,000 each In a wide variety of

vertebrates, three granular cell types (termed "A," "B," and "D") constitute the islet tissue There is evidence for assigning the insulin production to the B cells, which compose the major part of the islets These cells alone are found to degenerate when the islet tissue in the pancreas of partially depancreatized dogs is exhausted by carbohydrate overfeeding For additional information on the histology and pathology of the pancreatic islets the reader is referred to recent reviews by Gomori (65,66)

Diamare (40) in 1889, and Laguesse (106) in 1893, were probably the first to suggest that the islet tissue is concerned in the production of an internal secretion whose function is the control of carbohydrate metabolism Schulze (155) in 1900, and Ssobolew (167) in 1902, found that when they blocked the pancreatic duct with paraffin, the resultant sclerosis led to the destruction of the acinar tissue but left the islets unimpaired, and observed no symptoms of diabetes Many other investigators also noticed that although the gland atrophied after the ducts had been ligated, the islet tissue continued to function and diabetes did not occur When, on the other hand, the atrophied gland was removed, diabetes at once resulted Warthin (177) has given an excellent historical account of the discoveries which established the endocrine function of the islet tissue

After the discovery of insulin, Macleod's studies (120) definitely established the fact that insulin is elaborated only by the islet tissue This work was done on teleostean fish (hake, cod, pollock, haddock, etc) in which the islets are anatomically distinct from the acinous tissue Acid alcohol extraction of the islets yielded relatively large amounts of the hormone, while similar treatment of the zymogenous tissue yielded no insulin

Needham (132), studying the carbohydrate metabolism of the developing chick, found that the formation of the islet tissue in the pancreas is simultaneous with the glycogenic functioning of the liver

Homans (77) and Allen (2) showed that removal of a large part of the pancreas from dogs led to diabetes Kauer and Glenn (97) found it necessary to remove 84% of the pancreas from dogs before diabetes developed Houssay and his associates (81) have reported similar observations

More than fifty years ago Minkowski (127) and Weintraud (179) made the suggestion that the occurrence of glycosuria following pancreatectomy is associated with the food habits of the animal They found that, while pancreatectomy in the duck, chicken, or pigeon does not result in glycosuria, carnivorous birds like the hawk, falcon, buzzard, and raven on the other hand develop glycosuria and hyperglycemia on

removal of the pancreas These findings have recently been substantiated and extended by Mirsky and his associates (130,133)

IV. The Preparation of Insulin

Following the demonstration by von Mehring and Minkowski (122) in 1890 that the pancreas plays a role in the control of carbohydrate metabolism, many attempts were made to prepare an active extract of the pancreas which would alleviate the symptoms of diabetes mellitus The credit for the preparation of a pancreatic extract capable of effecting a lowering of the blood and urinary sugars, and serviceable in mitigating the symptoms of experimental diabetes in animals, and of human diabetes, belongs to Banting, Best, Macleod, and Collip The first active extract was prepared in 1922 in the following way after the pancreatic ducts of several dogs had been ligated, the animals were kept for a period of several weeks to allow the acinar tissue of the pancreas to degenerate This was done in order to circumvent the destructive action of the pancreatic enzymes on the hormone At the end of several weeks the degenerated pancreatic tissue was removed, sliced, and extracted with Ringer's solution The filtrate was found capable of reducing the hyperglycemia and glycosuria of depancreatized dogs (6) The name "insulin" was assigned to the active principle present in the pancreatic extracts It is interesting to note that, much earlier, de Meyer (123) in 1909, and independently Sharpey-Schafer (161) in 1916, had proposed the term "insulin" to designate the internal secretory product of the islet tissue of the pancreas

Since the initial successful preparation of insulin, various improvements in procuring therapeutically serviceable extracts have been introduced by other workers Detailed descriptions of the various methods employed in the preparation of insulin may be found in several reviews (55,76,80,88,157)

Practically all of the procedures are based on the extraction of the minced pancreas with acidulated or alkaline solutions, aqueous, acetone, methyl or ethyl alcohol Acid or alkaline-aqueous extractions have been found impracticable, especially for large-scale production Purification has generally been achieved by fractional precipitation with alcohol, by isoelectric precipitation, by salting out, by adsorption, or by the separation of the hormone as an insoluble salt Commercial insulin is at present prepared from the pancreas of beef or pig

Crystalline Insulin

Following the preparation of potent extracts of the hormone from the pancreas, numerous efforts were made to isolate the active principle as a

crystalline chemical entity Insulin was first obtained in crystalline form from highly purified commercial preparations by Abel and his associates (1) in 1926 Their method for obtaining crystalline preparations consisted of the isoelectric precipitation of the hormone from a strongly buffered acetic acid solution by the addition of a weak base The pH of the final solution was found to be about 5 6 Crystalline insulin was found to exhibit all the properties of a typical protein The isolation of insulin in crystalline form is probably the first instance in which a protein possessing a specific physiological action has been obtained in crystalline form

Modified procedures for obtaining crystalline insulin preparations have been worked out by several investigators (55,76,80,88,157) Addition of certain metal ions such as zinc, cobalt, nickel, and cadmium was found to facilitate greatly the formation of insulin crystals Crystalline insulin is now prepared on a commercial scale and is readily available (148) Although crystalline insulin is usually prepared from beef pancreas, it has also been obtained from the islet tissue of certain fish (92), from pig and sheep (156), and from bison and human pancreas (159) The crystalline preparations obtained from these various sources were found to possess the same maximal activity, 24 international units per mg , which remained constant on repeated recrystallizations, and to have the same sulfur content, approximately 3 3% It is pertinent to note here that insulins derived from various species have been found to be immunologically identical (178) Whether or not the active principle isolated from the pancreas represents the circulating natural hormone is still undetermined

V Chemistry of Insulin

Knowledge of the chemistry of insulin has not advanced greatly in the past few years Earlier reviews (52 55,76,80,88,89,91,157,173,181) on the chemistry of insulin are still intrinsically up to date For this reason the present discussion will be limited to a brief outline of present knowledge of the chemistry of the hormone

Solutions of insulin are levorotatory, as are those of all proteins The absorption spectrum of insulin can be accounted for by the tyrosine and cystine present in the molecule (37) Insulin does not show in its near-infrared absorption any difference in selective absorption from that shown by other proteins (8) The isoelectric point of insulin has been established at pH 5 3–5 35 (183) X ray studies of insulin have given little information as to the structure of the molecule, the patterns being similar to those obtained with other crystalline proteins (38)

It has been observed that crystalline insulin, prepared by different

methods, contains zinc The zinc content of the human pancreas has been found to range from 18 5 to 30 4 mg /kg of fresh gland Electrometric titration showed that the complex formed by zinc and insulin is analogous to that formed by zinc and glycine The zinc content of insulin varies with the preliminary treatment of the protein (49) Cohn and his associates (29), employing radioactive zinc, have determined the zinc content of insulin, crystallized in various ways They found that the amount of zinc varies from 0 3% to 0 6%, depending on the pH of crystallization Crystalline insulin with a low zinc content (0 15%) has been prepared by Sahyun (153) The optical crystallographic properties of crystalline "zinc insulin" have been reviewed by Keenan (98)

Crystalline insulin, studied by the Tiselius moving-boundary electrophoretic technique, was found to be homogeneous However, various amorphous insulin preparations of lesser potency, as low as 16 units per mg , have been found to be indistinguishable from crystalline insulin (71) This illustrates the well-recognized limitation of this method in determining the homogeneity of a protein preparation

Solubility of insulin in various solvents and its dielectric properties have been investigated by Cohn and his associates (29) I ens (109a) has determined the solubility curve of insulin samples in a sodium acetate-acetic acid buffer of pH 4 95 The molecular weight of insulin estimated from data obtained on redetermination of ultracentrifugal sedimentation and diffusion constants of carefully recrystallized insulin was 46,000 as against the previously found 36,000 (124,125)

A Amino Acids in Insulin

Since crystalline insulin was found to be a protein, investigations on the individual components, obtained on hydrolysis, were carried out with the object of determining whether insulin contained amino acids of unknown composition or whether constituents other than amino acids were present in the molecule The amino acids found to be present and their percentage are given in Table I

With the exception of serine and threonine, all other amino acids given in the table actually have been isolated and identified from the hydrolytic products of insulin The percentages of tyrosine, cystine, arginine, histidine, lysine, proline, and phenylalanine have been determined either by colorimetric methods or by calculation from the Van Slyke nitrogen distribution (52,55,76 80,88,89 157) Chibnall (28) has reported a value of 10 7% for histidine The presence of serine and threonine is based on the finding of Nicolet and Shinn that hydroxy-amino acids on treatment with periodic acid evolve ammonia (134) The content of glutamic acid (112,135) and of leucine (20,151) has been deter-

mined by microbiological methods The presence of alanine in insulin has recently been reported (28a,101a) Hydroxyproline seems to be absent, and it appears therefore that the nonamino nitrogen as found in the Van Slyke nitrogen distribution is present in the form of proline No evidence of the presence of aspartic acid has as yet been obtained, and it therefore appears that the amide nitrogen, as found in the Van Slyke nitrogen distribution, is present in the form of glutamine Tests for tryptophan have given negative results but indication of the presence of isoleucine and of valine in the crude leucine fractions has been obtained (90) No evidence has been found thus far for the occurrence in the

TABLE I

AMINO ACIDS IN INSULIN

Amino acids	Per cent	Method of determination
Tyrosine	12	Colorimetrically
Cystine	12	Colorimetrically
Arginine	3	Colorimetrically and calculated from Van Slyke nitrogen distribution
Histidine	4	Colorimetrically and calculated from Van Slyke nitrogen distribution
Lysine	2	Calculated from Van Slyke nitrogen distribution
Proline	10	Calculated from nonamino nitrogen of Van Slyke nitrogen distribution
Glutamic acid	17 5	Microbiologically
Leucine	13 5	Microbiologically
Threonine	2 6	Oxidation with periodic acid
Serine	3 6	Oxidation with periodic acid
Phenylalanine	7 0	Colorimetrically
Alanine	4 7	

insulin molecule of any constituent differing in its structure from the known amino acids

The number and nature of the free amino groups of insulin have been determined by the employment of 2,4-dinitrofluorobenzene It was found that an insulin submolecule of molecular weight of 12,000 contained two glycine and two phenylalanine residues containing free α amino groups and two lysine residues containing free ϵ-amino groups (153a) Jensen and Evans (91a) have previously shown by a related method that the amino groups of phenylalanine in insulin are free These results suggest that the insulin submolecule is made up of four open polypeptide chains, two of these having terminal glycyl residues and the other two terminal phenylalanine residues, the chains being bound together most probably by —S—S— linkages

B Chemical Modifications and Derivatives

Insulin can be inactivated and reactivated by bringing about and then reversing certain structural changes Structural changes can be obtained without inactivating the hormone By focusing the chemical attack on different points of the architecture of the molecule, it should be possible to determine whether insulin activity results from some force emanating from essentially the whole structure or from a specific field of force localized at some definite position or positions

C Sulfur

Of particular interest is the high sulfur content of insulin, approximately 3 3% In recent years it has been found that certain other hormones of protein nature also contain high amounts of sulfur It has been established that all the sulfur of insulin is present as a disulfide linkage and can be accounted for as cystine (175)

Reduction of the disulfide linkages in the insulin molecule under various experimental conditions results in a loss of physiological activity (52,55,76,80 88,157) It has been found that no proportionality exists between *maximal reduction and physiological activity, and that total* inactivation occurs with the reduction of approximately one third of the total sulfur Freudenberg and Munch (53) claim to have been able to produce some reactivation of cysteine-reduced and partially inactivated insulin by the addition of hydrogen peroxide However, experiments of other investigators have shown that re-oxidation of the reduced insulin does not restore its biological activity (52,89)

According to *Miller and Anderson (125,126), the primary change* in properties of the insulin on reduction with thioglycolic acid at pH 7–7 5 consists in an aggregation of the reduced molecules to form particles of much greater size than the original protein

D Alkali

Insulin, on treatment with alkali, is irreversibly inactivated with the *simultaneous liberation of ammonia and hydrogen sulfide (55,76,80,88,* 157) Freudenberg and Munch (53) have stated that, if insulin is heated in a solution of pH 10 5 for 15 hrs at 30°C , inactivation occurs without the liberation of ammonia and hydrogen sulfide or the appearance of sulfhydryl groups, no reactivation can be achieved Lanthionine has been isolated from insulin treated with dilute alkali (174)

E Acid and Enzymes

Hydrolysis of insulin by either acid or proteolytic enzymes leads to irreversible inactivation The failure of earlier attempts to prepare an

active extract from the pancreas was mainly due to the destructive action of the proteolytic enzymes present in the organ Inactivation by hydrolysis rapidly precedes the splitting of all peptide linkages

When insulin is heated with $N/10$ hydrochloric acid at 100°C, a "heat precipitate" is formed which is physiologically inactive Simultaneously ammonia is liberated, probably arising from the amide group of the glutamine portion of the molecule On treatment of the precipitate with dilute alkali, a product is obtained which exhibits approximately 80% of the physiological activity of the original material (55,76, 80,88,157,173)

F Acetylation

By the treatment of insulin with acetic anhydride or ketene in the cold, acetylated products with greatly diminished activity are obtained The inactivation is partially reversible since on hydrolysis of the acetylated insulin with weak alkali a substance more active than the acetylated compound, but less active than the original insulin, is obtained (55,76 80,88,157 181)

Ketene is well suited for the acetylation of a protein because it is possible to work in aqueous solution and at low temperatures It has been found that this reagent reacts much more rapidly with free amino groups than with the hydroxyl groups of the protein molecule

G Acid Alcohol

Insulin when allowed to stand in acid alcohol for several hours, is converted into a relatively inactive product On treatment of this compound with very dilute alkali about 60% of the original activity is restored A part of the reactivated material may be recovered in a crystalline form identical with that of crystalline insulin Scott and Fisher (157) have shown that inactivation also occurs with such organic solvents as acetone, in the presence of certain amounts of hydrochloric acid Inactivation therefore cannot be due to esterification but probably involves a reversible intramolecular rearrangement (55,76,80,88,157)

H Iodine

Iodine in faintly alkaline solution was found to inactivate insulin irreversibly in a short time, probably due to oxidation (55,76,80,88,157) Insulin iodinated according to the method of Neuberger is assumed to yield a product in which the hydrogen of tyrosine is substituted by iodine in the 3,5 positions The iodinated insulin was found to retain only 5–10% of the original activity, partial removal of the iodine by

catalytic reduction was accompanied by an approximate proportional restoration of activity (72)

I Miscellaneous Experiments

Several azo derivatives of insulin containing up to fifteen azo groups per molecule were prepared by Reiner and his associates, two of the azo compounds were obtained in crystalline form The activity of the hormone was somewhat impaired by positively substituted azo groups and less impaired by those containing negative substituents (107,141) Complexes of insulin with piperidine and primary bases have been obtained in crystalline form (160)

Treatment of insulin with either aliphatic or aromatic aldehydes or isocyanates in weakly alkaline solution was found to yield insulin derivatives retaining only a small percentage of the physiological activity of the hormone Chemical reaction between either amino or hydroxy groups of the insulin molecule and the reagent used can take place under the experimental conditions employed (55,76,80,88,157) The action of phenyl isocyanate on insulin has been studied in detail by Hallas-Moeller (71a)

The effects of various other reagents, such as methyliodide, diazomethane, and nitrous acid, on insulin have also been studied and found to yield physiologically inactive products (55,76,80,88,157)

Attempts have been made to interpret the results obtained on treatment of insulin with various reagents, as indicating that certain groups of the insulin molecule, such as phenolic hydroxyl, primary amino, and the disulfide linkage, are essential for the physiological activity of the hormone

The pharmacodynamic function of insulin may be due to

(I) The presence of a prosthetic group in the insulin molecule As already indicated, no evidence of such a group in the insulin molecule has yet been obtained

(II) The occurrence in the insulin molecule of an unknown specific amino acid Only known amino acids have thus far been isolated from the hydrolytic products of insulin

(III) The existence in the protein molecule of a specific grouping of certain component amino acids embedded in the molecule, and which by virtue of their chemical and spatial configuration impart a specific pharmacodynamic function to the protein molecule For this reason, the entire molecule is necessary for the physiological activity of the protein

It is the author's opinion that, from the evidence at present available and outlined, it must be assumed that the hypoglycemic activity of insulin is a specific property of the whole protein molecule Any reaction

which may produce a change in the architecture of the protein molecule is likely to cause a loss of physiological activity The complex protein structure of insulin permits little hope at present for the elucidation of the exact structure and for the synthesis of this hormone

VI. Standardization of Insulin

Up to the present, only biological methods of assay have proved applicable for the determination of the physiological activity of insulin Various suggested chemical methods of assay have been found nonspecific

The potency of an insulin preparation is expressed in international units, the unit being defined as "the activity contained in 0 125 mg of the international standard preparation" Recently a standard insulin preparation of crystalline zinc-insulin has been proposed by the National Institute for Medical Research in London A unit is $\frac{1}{22}$ mg of this preparation

Two procedures have been devised to determine the activity of insulin preparations by comparison with the standard and are now generally employed (I) a method dependent upon the production of convulsions, and (II) a method based upon the determination of the decrease in blood sugar

Briefly stated, method I, which employs mice as test animals, is based upon the comparison of the incidence of convulsions produced in white mice kept at 38°C , half of the mice being injected intraperitoneally with the standard preparation and the other half with the solution of unknown potency The mouse unit is defined as the quantity producing convulsions in one half the number of mice injected

Method II consists of injecting subcutaneously a suitable dose of the standard insulin preparation into one half of a series of rabbits of 2 kg weight and previously starved for eighteen to twenty-four hours, the other half simultaneously receiving a dose of the sample of unknown potency Several days later the groups are crossed over and used for the injection of the same preparations Blood samples are usually taken at *one and a half , at three-, and at five-hour intervals after the injections* From the relation between the lowering of blood sugar produced by the standard insulin and that produced by the insulin of unknown potency, the activity of the latter can be calculated A few recent references on the standardization of insulin are included in the bibliography (15,16,25,50,51,87,162)

Gellhorn and associates (56) have described a sensitive method for the assay of insulin based on the use of adrenodemedullated-hypophysectomized rats In such animals administration of insulin in amounts of 0 001 units per 100 g of body weight may cause convulsions and coma

Employing this method these investigators estimate that the normal content of insulin in human blood is 0 002 units per ml Opdyke (136) has described a method for the biological assay of insulin based upon the blood sugar response of the fasting chick

VII Administration of Insulin

Since insulin is of proteinlike nature the hormone has no effect when taken orally as it is inactivated by the proteolytic enzymes (pepsin and trypsin) and therefore must be administered parenterally This constitutes one of the chief difficulties and objections to its use While administration of insulin remains effective in spite of repeated injections, other protein hormones, such as the anterior pituitary principles, become progressively less effective on continued administration

Clinical assays conducted on patients with uncomplicated diabetes on certain standard dietary regimens, reveal that one insulin unit will on an average promote the metabolism of approximately 1 5 g of glucose

Insulin is usually administered to diabetic patients by the subcutaneous route, and sometimes in cases of emergency by intravenous injection There are associated with these generally accepted methods of administration certain practical difficulties which investigators have recognized for many years and attempted to remedy Chief among these difficulties are the discomfort accompanying injection, and the frequency of dosage with its attendant inconvenience

Since the parenteral injection of regular insulin causes wide fluctuations in blood glucose levels and requires several daily injections, experiments were carried out with the object of combining or mixing insulin with certain substances in order to decrease its rate of absorption from the tissues Such delayed absorption would permit the use of larger doses and thus reduce the number of daily injections required Hagedorn and his associates (67) were the first to produce an insulin preparation possessing prolonged blood sugar-lowering action and suitable for therapeutic use These investigators demonstrated that when a solution of protamine in sodium phosphate buffer is added to an insulin solution, an insulin protamine complex is formed at pH 7 2, which on subcutaneous injection produces prolonged hypoglycemia

Shortly after Hagedorn's finding, Scott and Fisher (158) reported that the addition of zinc as a salt to insulin prior to the addition of protamine prolonged still further the hypoglycemic effect of the hormone The hope that a single injection daily of protamine zinc insulin would suffice to establish good control in the majority of diabetics unfortunately has not been realized, only the milder cases can be so regulated

For practical purposes a combined form of insulin therapy is often

employed two separate injections are given each morning, one of protamine-zinc insulin and one of regular insulin It is obvious that this mode of duplicate administration is not entirely satisfactory

Efforts have therefore been made to obtain an insulin preparation which, with a single injection daily, would establish good control in severe as well as mild cases First, proteins other than protamine, such as globin and histone, have been combined with insulin Apparently neither of these modifications seems to offer any significant advantage over protamine-zinc insulin Second, modifications of protamine-zinc insulin have recently been suggested (I) an insulin preparation containing about 25% of the hormone in quickly absorbable form, and 75% in precipitated, slowly absorbable form, designated "modified protamine-zinc insulin" by MacBryde (118,119), and (II) an insulin preparation which is obtained by mixing crystalline insulin with protamine-zinc insulin, as suggested by Colwell (31) Reference is made to several comparative clinical studies of the more recently developed insulin preparations (5,9,14,31,39,48,109,117,118,119,121,131,138,145,172) Attempts to prolong the action of insulin by implantation of "insulin tablets" under the skin have not proved encouraging (184)

The absorption rates of different forms of insulin which were labeled with radioactive iodine have been studied in rabbits (140,142) The decrease in radioactivity at the site of injection was the index of the rate of absorption The order of rapidity of absorption was regular insulin, globin insulin, and protamine-zinc insulin Employing the same procedure the rate of absorption of insulin in human patients has been studied In uncomplicated diabetes the absorption rate of insulin was normal but patients with idiopathic insulin resistance showed a significant delay in absorption (149)

In patients totally pancreatectomized for the removal of carcinomas, the insulin requirement was found to be very small, about 40 units per day (62,146) This observation is in agreement with the finding that dogs with 90 to 94% of the pancreas removed seem to require more insulin than *completely pancreatectomized dogs (44)* According to Lerman (111) insulin resistance is dependent upon the appearance and concentration in the body of antibodies to insulin

VIII Physiological Action of Insulin

Insulin plays an important role in the regulation of various phases of metabolism Metabolism is commonly defined as the sum total of the chemical changes which occur in the various tissues of the body The distribution and excretion of inorganic ions is referred to as "inorganic metabolism" while the turnover of carbohydrate, fat, and protein is

referred to as "organic metabolism" The different phases of carbohydrate, protein, and fat metabolism are intimately linked together

It is perhaps advisable to present at this point a brief description of the main phases of carbohydrate metabolism in normal animals Glucose is the sugar which is most efficiently metabolized by the tissues of the organism and other sugars are generally converted into glucose by the liver before they can be utilized Certain amino acids present in proteins can be converted into carbohydrates, and carbohydrate can be converted into fat in the body The possibility of a conversion of physiologically important amounts of fat into carbohydrate, however, has not yet been definitely proved Foodstuff passes through a common metabolic pool and in this sense all three foodstuffs are interconvertible

Absorbed sugar is either oxidized in the tissues or converted into glycogen or fat The oxidation of glucose and the synthesis and breakdown of glycogen are evidently rapid and constantly occurring processes Glucose is burned for energy in the various tissues of the body Muscle glycogen is oxidized to lactic acid, thus furnishing part of the energy for muscular activity Some of the lactic acid is transformed into glycogen in the liver

The concentration of glucose in the blood is of importance in supplying the various cells with sugar The liver is the organ of major importance in the regulation of the blood sugar level, in its absence the blood sugar rapidly falls It is the function of hepatic glycogen to maintain the blood sugar level, muscle glycogen is not a source of blood sugar

The blood sugar level represents the resultant of oxidation, storage, and excretion on the one hand, and of formation and absorption on the other Hyperglycemia may result from (a) excessive carbohydrate intake, (b) inadequate carbohydrate utilization, (c) carbohydrate over production Conversely, hypoglycemia may be due to (a) starvation, (b) excessive carbohydrate utilization, (c) inadequate carbohydrate formation These extremes of glycemia act as stimuli to the regulatory mechanisms, which in turn tend to reestablish the normal blood sugar level The efficiency with which these regulatory mechanisms can counteract the extremes of glycemia depends in a large part on the normal endocrine balance (164)

Metabolism is controlled by the proper physiological coordination of various active agents in the body, hormones, vitamins, and enzymes may be classified as such agents The efficiency with which the mechanisms of the endocrine system counteract each other in regulating metabolism depends to a large extent upon normal endocrine balance Any relative or absolute deficiency or preponderance of certain endocrine secretions may result in a definite abnormal shift of general metabolism Cog

nizance should also be taken of whether or not any specific physiological change observed is an immediate manifestation of those reactions into which the hormone enters in order to produce a certain physiologic response Apparently hormones do not initiate any new metabolic processes but rather influence the rate of speed of existing processes by accelerating or inhibiting certain enzymatic reactions in the cell upon which they act

The influence of the various endocrine secretions on metabolism is briefly outlined The exact role which each of the different endocrine principles plays in the process of metabolism has not been fully estab lished as yet

A Pancreas

The importance of insulin with regard to metabolism becomes evident upon examination of the physiological disturbances in the body which are observable in the absence of the secretion of insulin (pancreatectomy, diabetes mellitus) The following symptoms have been found to be characteristic

(1) Pronounced hyperglycemia and glycosuria

(2) Depletion of the glycogen stores in certain tissues (liver, muscle)

(3) Lowering of the respiratory quotient, indicating a decrease in the rate of the oxidation of glucose

(4) Increase in the NPN of the urine, which is due to an increase in the conversion of protein into glucose

(5) Increased formation of ketone bodies (ketosis), caused by an acceleration of fat catabolism

Injection of insulin will relieve all these symptoms and re-establish a practically normal metabolism

B Pituitary (78,114,185,186)

Injection of a posterior pituitary extract causes a diminution of liver glycogen but effects no change in muscle glycogen Knowledge of the action of the anterior pituitary on metabolism is still incomplete While some of the principles (growth and lactogenic) of this endocrine organ act directly on the tissues, others (thyrotrophic, adrenocorticotrophic and gonadotrophic) exert their influence by stimulating their respective end organs (thyroid, adrenal cortex, and gonads)

That the anterior pituitary influences metabolism is shown by the following observations

(1) Removal of this organ renders the animal more sensitive to insulin The glycogen stores of the liver and muscles are more rapidly depleted on fasting than in normal animals Injection of anterior

pituitary extracts prevents this depletion of the glycogen stores and renders the animal more resistant to insulin Hypophysectomized animals show a decreased excretion of nitrogen in the urine, indicating a decreased breakdown of body protein A decrease in the total metabolism of the body is also observed

(2) The symptoms of experimental diabetes (removal of the pancreas) are greatly ameliorated following hypophysectomy The diabetic symptoms are manifested again upon injection of anterior pituitary extracts into such doubly operated animals

(3) A diabeteslike effect can be produced in normal dogs by the daily injection of increasing amounts of anterior pituitary extracts over a period of several days or weeks The same effect can be obtained more readily in partially depancreatized animals

C Adrenal (84,85,99)

In general it may be said that the influence of this endocrine organ is similar to that of the anterior pituitary, since part of its functional integrity is under the control of the anterior pituitary

The principle of the adrenal medulla accelerates glycogen breakdown in the liver and muscle tissue

The function of the adrenal cortex may be classified into two main groups

(1) Control of the distribution and excretion of inorganic ions

(2) Control of organic metabolism

The adrenocortical principles which influence primarily the distribution and excretion of electrolytes are not the same as those that affect primarily organic metabolism

The effects of those adrenocortical hormones that influence organic metabolism may be due to

(1) An inhibition of the peripheral utilization of glucose

(2) An inhibition of liver glycogenolysis or an acceleration of the conversion of glucose to glycogen

(3) A control of the rate of deamination of amino acids and in consequence of the rate of gluconeogenesis from protein

D Thyroid (79)

The rate of oxygen uptake of all tissues is increased following the injection of the thyroid hormone Prolonged administration of the thyroid principle produces diabetes in partially depancreatized dogs

E Gonads (101)

The relation of the secretion of the various sex glands to metabolism has not yet been well defined

For a more detailed description of the metabolic influence of the pituitary, adrenal, thyroid, and gonads the reader is referred to the chapters in this monograph dealing specifically with these endocrine glands It is apparent from the foregoing brief outline of the influence of the secretions of the various endocrine organs on metabolism that the physiological action of insulin is in general antagonistic to that elicited by the pituitary, adrenal, and thyroid (113)

IX Endocrine Function of the Pancreas

In the following paragraphs the endocrine function of the pancreas will be briefly discussed It is not within the scope of this review to discuss all the publications dealing with the physiological action of insulin and hence only those which appear to reflect the major trends of more recent work on this subject will be considered For a discussion of the earlier investigations on this subject the reader is referred to various reviews (55,76,80,88)

Pancreatic endocrine function has been studied from several points of view (a) the effect of various factors on the insulin content of the islet tissue of the pancreas, (b) the control of the secretion of insulin, (c) the physiological effect of insulin

According to Haist (69), who reviewed the factors influencing the insulin content of the pancreas there are two types of processes by which the amount of insulin in the pancreas may be reduced those which decrease the need of insulin and so reduce its production, as fasting, high fat diets the administration of insulin, and those which increase the need for insulin relative to the available supply, as partial pancreatectomy, treatment with diabetogenic anterior pituitary extracts, probably also treatment with adrenocortical extracts since Ingle (83,86) has shown that glycosuria and hyperglycemia can be produced in normal rats by the administration of large amounts of 17-hydroxycorticosterone and 17 hydroxy-11 dehydrocorticosterone Insulin injections enhance the effects of fasting and fat feeding in the rat, reducing the insulin content of the pancreas to very low values Experimental evidence seems to support the view that fasting fat feeding and insulin administration reduce the need for endogenous insulin and lower the insulin content of the pancreas by making the islet cells less active (69) On the other hand, administration of insulin tends to prevent the lowering of the insulin content or the islet changes that may be observed following anterior pituitary injection or partial pancreatectomy

Allen (2) using partially depancreatized dogs showed that the islets developed hydropic degeneration within a week after sugar was found in the urine This change progressed until the fourth to sixth week of

glycosuria Thereafter the islets underwent atrophy, becoming few and small Copp and Barclay (33) demonstrated morphological recovery of hydropic islets in partially depancreatized dogs during treatment with insulin Although the hydropic islets were restored, recovery of the animals was not possible because the pancreatic remnants had been originally too small Bell, Best, and Haist (10) reported that in partially depancreatized dogs, when a sufficient amount of the gland was removed to produce diabetes, the insulin concentration in the remnant was reduced to very low values, whereas when enough gland was left to prevent the onset of diabetes, the insulin concentration in the remnant was usually found to be within the expected normal range These findings support the previously expressed view of Allen (2) that the degenerative changes which occur in the islet cells of the diabetic partially depancreatized dog result from overstimulation of the insulin secreting mechanism Mirsky and his associates (129) observed that in partially depancreatized dogs a persistent diabetic condition could be produced by excessive and prolonged insulin administration Gellhorn Feldman, and Allen (57) could not detect any insulin in the blood of totally depancreatized dogs

Following the observation of Young and his associates (144) that permanent diabetes could be produced in dogs by the injection of anterior pituitary extracts and that this was associated with destruction of the islet tissues, Best and his associates (13) have shown that daily administration of insulin along with the anterior pituitary extract tends to prevent reduction in the insulin content of the pancreas and degranulation and hydropic degeneration in the beta cells of the islet tissue Lukens and Dohan (115) have made the interesting observation that the diabetes produced in partially pancreatectomized cats by administration of anterior pituitary extracts may be allowed to continue for several weeks, after which, if the animals are adequately treated with insulin for a few weeks, the subsequent withdrawal of insulin is not followed by glycosuria Indeed, the animals may be said to have recovered from the diabetes, and this conclusion was borne out by histological examination of the islet tissue, which showed that the characteristic hydropic degeneration present prior to insulin treatment had disappeared

From these and other experiments it appears that treatment with insulin can improve both anatomically and functionally the islets damaged by anterior pituitary extract, if the lesions have not advanced too far According to Lukens (116) hyperglycemia is the chief causative factor in the subsequent failure of the pancreas, and prevention of hyperglycemia by insulin protects the islet tissue Hyperglycemia probably leads to an exhaustion of the beta cells in the islet tissue through overwork

The view that diabetes mellitus is due solely to failure of the pancreatic islet cells to secrete adequate quantities of insulin is not very satisfactory in the light of the finding that a diabetic may display an apparently normal pancreas Mirsky (128a) has proposed the thesis that diabetes mellitus in man is due to an insufficiency of insulin which is only rarely due to a decreased production of insulin by an inadequate pancreas Mirsky proposed that in most instances of human diabetes there is an increased utilization, destruction, or inhibition of insulin by tissue proteinases or by an insulin antagonist which results in a decrease in the concentration of circulating insulin The possibility of a dysfunction of the mechanism which controls the release of the active principle from the cells in which it is formed may also have to be considered

The prevention and cure of diabetes in experimental animals may arouse hope that similar procedures may some day be applicable to the human subject However, this will not be easy until the potential human diabetic can be recognized much earlier than is possible at present, and until more information becomes available on the etiology of the diabetic state in man Lukens (116) has reviewed the clinical and experimental data relating to the etiology of diabetes mellitus (See also ref 176)

A Control of Insulin Secretion

The present evidence seems to indicate that the production and secretion of insulin is not directly dependent on endocrine factors since it has been found that hypophysectomized (68), adrenalectomized, and gonadectomized (70) animals do not exhibit any signs of insulin deficiency

It is generally assumed that the blood sugar level regulates the secretion of insulin There is some indication that the blood insulin level may be involved The chemical factor is the principal and essential mechanism, the nervous (vagal) factor is secondary and dispensable, and only acts as an accessory mechanism increasing the speed of the adjustment Conditions that depress the blood sugar level lessen the requirements for insulin while those that result in an elevation of blood glucose stimulate an increased insulin production A continued stimulation such as is produced by a continued high level of blood glucose appears to cause at least in some species an ultimate breakdown of the insulin secretory mechanism resulting in diabetes However in a recent communication Conn and Louis (32) claim to have obtained evidence of the presence in the anterior pituitary of an insulotrophic principle which directly stimulates the islets of the pancreas It is difficult to reconcile this view with the finding that hypophysectomy fails to cause any significant involution of the islets

B Alloxan Diabetes

It may be pertinent to refer here briefly to the effect of alloxan on the islet tissue of the pancreas In 1943 Dunn and McLetchie (47) had reported the interesting finding that parenteral administration of alloxan may be followed, in rabbits and rats, by complete necrosis of the pancreatic islet tissue, together with a condition of persistent hyperglycemia and glycosuria in which all the cardinal symptoms of diabetes mellitus may be manifest The glycosuria could be abolished by the administration of insulin These findings have been confirmed and extended by numerous investigators Histological studies of the pancreas after treatment with alloxan have revealed an immediate degranulation and later degeneration of the beta cells The alpha cells remain unaffected (63) This observation fortifies the hypothesis, derived from earlier studies of the pathological changes in diabetes, that insulin is secreted by the beta cells This observation also paves the way for studies of the possible separate functions of the component cells of the islet tissue Apparently alloxan affects the beta cells of the islet tissue directly and does not act through the medium of disturbed blood sugar regulation The degeneration of the beta cells consequently leads to a decrease of insulin production The reason for the necrotic effect of alloxan on the beta cells of the pancreatic islets is not known Dunn, Kirkpatrick McLetchie, and Telfer (46) have discussed the possibility that alloxan may be formed in the body under physiological conditions and may act as a regulator of islet activity They refer to alloxan as a "possible cause of an initial disturbance of the islet system which may eventuate in diabetes mellitus" However, direct evidence for such a biological role for alloxan is at present lacking For additional information the reader is referred to several reviews and reports on the mechanism of alloxan diabetes and on the pathological and metabolic changes obtained in various animals on treatment with alloxan (4,45,63a,64,82,93,100,150)

C The Effect of Insulin in Animals

There is general agreement on the interpretation of the physiological responses observed on administration of insulin in depancreatized animals restoration of blood sugar to normal, rise of the respiratory *quotient, inhibition of excessive ketogenesis and gluconeogenesis, and* adjustment of the glycogen stores to normal In connection with the changes in glycogen in depancreatized animals, Pauls and Drury (137) found, on administration of insulin in fasted depancreatized rats, a marked augmentation in muscle glycogen (0 16%–0 60%) and also in liver glycogen (3 26%–9 78%), which is in agreement with earlier work of other

investigators (55,76,80,88) According to Pauls and Drury (137) the increases in glycogen stores, however, account for only about one quarter of the glucose which is metabolized They suggest that one of the principal effects of insulin is the promotion of a conversion of glucose to fat

Administration of insulin also affects the carbohydrate metabolism in the normal animal, as indicated, for example, by the reduction in the blood sugar level The interpretation of the physiological responses, observed on administration of the hormone to normal animals, is however more complex than that in depancreatized animals The lowering of the blood sugar level under the influence of insulin is a result of the more rapid withdrawal of sugar from the blood by the other tissues Administration of insulin plus glucose to fasting normal animals causes a more pronounced rise in the R Q than does either glucose or insulin alone According to Soskin and Levine (165) the administration of insulin to the normal animal does not increase the utilization of carbohydrate in the organism as a whole Bridge (2 1)has reinvestigated the glycogenic effect of insulin and glucose administration in normal rabbits under well controlled conditions From these experiments it seems that, of the aspects of carbohydrate metabolism observed, the action of insulin is seen only in the distribution of glycogen between liver and muscle tissue, without insulin, a large proportion of infused glucose is deposited as glycogen in the liver, whereas under the influence of insulin relatively little goes to the liver, most of it appearing in the muscles This confirms earlier work of other investigators (17,18,34) The apparent contradiction that insulin injections do not increase glycogen deposition in the liver of normal animals can be explained by the effect of the blood sugar level on the reaction glucose $\rightleftarrows$ glycogen, which will go to the left if the blood sugar is below normal, a mechanism which is essential for the regulation of the blood sugar level Any insulin administered to a normal animal is in excess over the optimal amount already present, preventing liver glycogenolysis at first and then causing hypoglycemia, which stimulates the secretion of adrenaline, releasing glucose from liver glycogen The sugar released is deposited in the muscle as glycogen, the blood remaining normal or low Therefore we find that the glycogen stores of the liver either are not increased or are diminished after insulin administration to a normal animal, while the glycogen stores in the muscle are increased

In vitro studies have demonstrated that insulin enhances glycogen formation in muscle tissue The increased deposition of glycogen is not associated with an increased oxygen consumption nor with an increased respiratory quotient (58 59,73) Stetten and Klein (170), studying the formation of glycogen in the previously fasted rat in response to insulin

by the isotope technique, found that the glycogen appearing in the muscle after administration of insulin plus glucose is apparently formed largely directly from glucose

The diabetic organism excretes abnormally high amounts of nitrogen in the urine, indicating that insulin inhibits protein catabolism It has been reported that the transformation of certain amino acids into carbohydrate, observed in excised liver slices *in vitro*, is inhibited by insulin (3a,169a) The reduction of the amino acid concentration in the blood, observed after administration of insulin to normal animals, is secondary to an increased secretion of adrenaline produced by the hypoglycemia

Diet apparently has an influence on the sensitivity of the animal to insulin Roberts and Samuels (147) found that adult male rats force-fed a high-fat diet were less sensitive to the action of injected insulin than rats force-fed an isocaloric high-carbohydrate diet The decreased sensitivity was manifest as a markedly increased rate of recovery from insulin hypoglycemia The cause may have been the higher level of liver glycogen in the fasted fat-fed animals Gaebler and Ciszewski (54) observed that omission of yeast from the diet caused appearance of hyperglycemia and glycosuria in depancreatized dogs which were maintained with insulin Resumption of yeast feeding abolished glycosuria in about 12 days Biskind and Schreier (14a) reported that intensive and persistent oral, or oral and parenteral therapy with vitamin B complex in diabetes, showing symptoms of deficiency of factors of the B complex, led to striking improvement in general health and often to marked improvement in carbohydrate metabolism frequently with reduction in insulin requirement or its elimination altogether

D Intermediary Metabolism of Glucose

It has already been pointed out that synthesis or breakdown of glycogen, as well as oxidation or formation of glucose, are evidently rapid and constantly occurring processes which proceed in a series of steps, each under the control of a specific system of enzymes (Table II) For additional information on the intermediates of glucose metabolism the reader is referred to various reviews *(7,30,43,103,168)*

It has been found that the transformation of glucose and its intermediates as outlined in Table II can take place without the mediation of insulin in cellfree extracts and in some systems consisting solely of suitable substrates, enzymes, and their cofactors

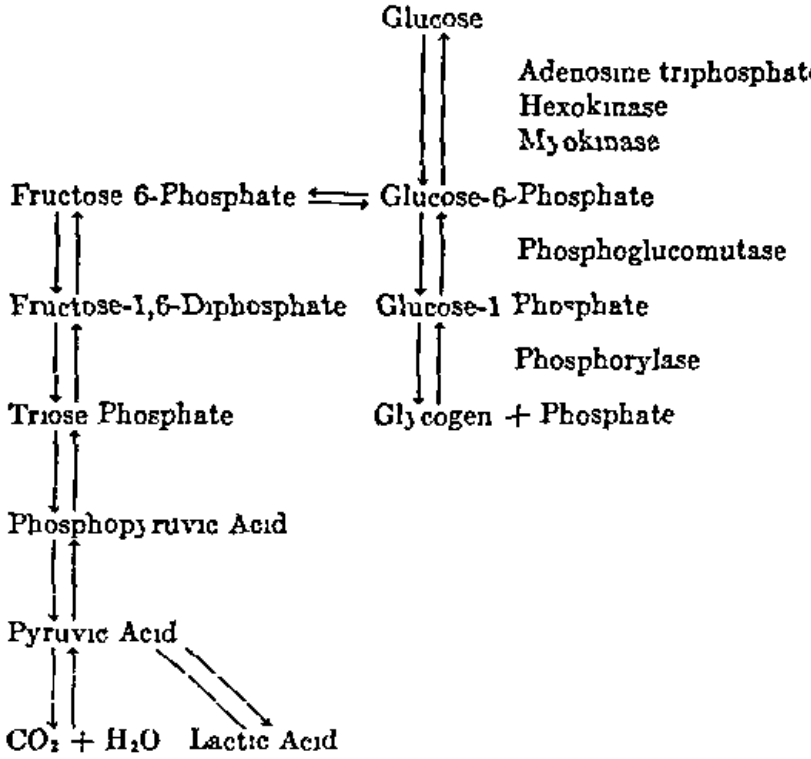
The question as to the way in which insulin exerts its physiological action is naturally of great interest It is difficult to conceive the hormone as reacting directly with the different metabolic substrates It seems more likely that insulin, and also the other hormones, while not

participating in the actual enzymatic processes, may accelerate or inhibit certain enzymatic reactions or may cause an increased concentration of a given enzyme in the tissue upon which they act Unfortunately, the mode of hormone action is a field which, in spite of its great importance, has remained almost unexplored thus far

When insulin is added to suspensions of pigeon breast muscle, the respiration of the tissue is maintained for a longer period of time than with other similar control suspensions (104) This effect has been shown

TABLE II

INTERMEDIARY CARBOHYDRATE METABOLISM*



* This scheme omits the detailed steps by which phosphate is transferred between the carbohydrates and the adenosine triphosphate and phosphocreatine systems, and also omits the tricarboxylic acid cycle operating between pyruvic acid and CO_2.

by Rice and Evans (143) to be reflected in the sustained ability of the insulin supplemented tissue to oxidize pyruvate When insulin is absent, practically no pyruvate is utilized after 60–90 minutes, although the oxygen uptake of the suspension is of considerable magnitude

The *in vitro* relationship between insulin and pyruvate oxidation shown by these experiments may be indirect, however, since results obtained in humans and dogs seem to exclude any participation of insulin in pyruvate oxidation (23,24,26) Bueding and his co-workers (23) have shown that pyruvic acid does not accumulate in the blood of the diabetic animal after the administration of glucose until insulin has been injected,

after which it appears in large quantities These findings indicate that insulin may act at some intermediary stage between glucose and pyruvic acid

Researches in recent years have demonstrated the importance of phosphate intermediates in carbohydrate metabolism (35,163,169) The influence of insulin and glucose administration upon several phosphate fractions of the blood, liver, and muscle of various animals has been investigated The diabetic organism exhibits an abnormally high level of inorganic phosphate in the blood, which is corrected by treatment with insulin Soskin Levine, and Hechter (166) reported that the reduction in the amount of inorganic phosphate in the blood found in normal animals on administration of insulin is due to insulin, while the rise in the hexose monophosphate content of the muscle observed under the same experimental conditions is due to adrenaline and results from the breakdown of muscle glycogen It is sometimes difficult to differentiate between insulin and adrenaline actions in normal animals, since hyperglycemia causes a stimulation of the pancreatic islets and hypoglycemia a stimulation of the adrenals These findings of Soskin, Levine, and Hechter have been substantiated by Weissberger, employing labeled phosphate (180) Administration of insulin apparently causes an accumulation of hexose monophosphate in the blood (95 180) Using radioactive phosphorus as a tracer, Sacks (152) observed that injection of insulin into cats caused a marked increase in the turnover rates of phosphocreatine and adenosine triphosphate in resting muscle during glucose absorption, but there was no increase in the turnover of glucose-6 phosphate beyond that produced by glucose alone Sacks postulated that the increased turnover was associated with the increased oxidation of glucose which insulin produced in resting muscle Phosphate in the liver was found by Kaplan and Greenberg (94,95) to be increased at the expense of the plasma inorganic phosphate after injection of insulin to rabbits given radioactive phosphate Both insulin and glucose caused an increase in the radioactive adenosine triphosphate and a decrease in the residual and alcohol soluble radioactive phosphate of the liver Injection of malonate inhibits the degree of increase in liver adenosine triphosphate which follows the injection of insulin Coincident with the lower adenosine triphosphate values there is an increase in inorganic phosphate (96)

Stadie (169), reviewing the problem as to whether insulin affects the metabolism of phosphate, concluded "There is good evidence that insulin plays a role in the metabolism of phosphate This is particularly true when the interrelations of carbohydrate and phosphate metabolism are considered The precise chemical mechanisms by which this effect of insulin is brought about are however far from elucidated"

The recent finding of Price, Cori, and Colowick (139) that the inhibitory effect of certain anterior pituitary fractions in the transformation of glucose to glucose 6 phosphate could be released by insulin, is of great interest in this connection As can be seen from Table II, the first step in the utilization of glucose by animal tissues, a step common to its transformation to glycogen or its oxidation, is the formation of glucose-6-phosphate The action of insulin in the tissues, therefore, is to promote the conversion of glucose into glucose-6-phosphate, an intermediate substance which is necessary for both utilization and glycogenesis

Employment of labeled agents such as radioactive phosphorus and the isotopes of carbon, nitrogen, sulfur, and other elements will, no doubt be of great help in obtaining a more precise knowledge concerning the mode of action of insulin

From experimental data at present available, the following functions may be attributed to insulin

(1) Acceleration of glucose oxidation in the tissues

(2) Increase in the rate at which glucose is converted to glycogen and fat in the various tissues It is still undetermined whether insulin has a direct influence on the formation of liver glycogen or whether it inhibits hepatic glycogenolysis which is caused by certain other hormones This inhibitory effect of insulin would enable the liver of the normal animal to retain its glycogen, and would also account for the disappearance of liver glycogen in the depancreatized animal (absence of insulin)

Increase in glucose oxidation and in the rate of glycogen formation probably accounts for the fall in blood sugar observed after insulin injection in depancreatized animals

(3) Inhibition of carbohydrate formation in the liver from noncarbohydrate sources Gluconeogenesis is under the partial control of certain other endocrine principles (anterior pituitary and adrenal cortex)

(4) Inhibition of excessive formation of ketones

Decrease in glucose oxidation and increase in hepatic glycogenolysis and gluconeogenesis cause hyperglycemia, which may be due to the following factors

(a) Deficient supply of insulin

(b) Liberation either at a normal or excessive rate, of those principles which enhance glycogenolysis and gluconeogenesis, and of those which reduce glucose utilization

Two apparently opposing views regarding the mechanism of the metabolic actions of insulin on the one hand and the secretions of the anterior pituitary, the adrenal cortex, and the thyroid on the other hand, have been expressed

I Nonutilization theory In the absence of insulin, the capacity of peripheral tissue to metabolize glucose is greatly diminished Furthermore, certain of the anterior pituitary and adrenal cortical principles depress the utilization of glucose and in addition either directly or indirectly stimulate glucose production in the liver from protein

II The overproduction theory, on the other hand, postulates that glucose utilization in the peripheral tissue is not influenced to a great extent by insulin or by the anterior pituitary and adrenal cortical principles The excess amount of glucose is due solely to a stimulation of increased glucose production not only from amino acids but also from fatty acids in the liver by certain of the anterior pituitary and adrenocortical hormones The overproduction theory has been mainly developed by Soskin and his associates (165a)

The two opposing views are not necessarily mutually exclusive, but each, to a lesser or greater degree, may constitute one aspect of a more comprehensive understanding of metabolism

REFERENCES

1 Abel, J J , Geiling, E M K , Rouiller, C A , Bell F K , and Wintersteiner, O *J Pharmacol* **31**, 65 (1927)
2 Allen, F M *J Metabolic Research* **1**, 5 53, 75 89 (1922)
3 Allen, F M *ibid* **1**, 5 (1922)
3a Bach, S J , and Holmes E C *Biochem J* **31**, 80 (1937)
4 Bailey, C C , Bailey, O T , and Leech, R S *New Engl J Med* **230**, 533 (1944)
5 Bailey, C C , and Marble, A *J Am Med Assoc* **118**, 683 (1942)
6 Banting, F G , and Best, C H *J Lab Clin Med* **7**, 464 (1921–22)
7 Barron, E S G *Advances in Enzymol* **3**, 149 (1943)
8 Bath, J W , and Ellis, J W *J Phys Chem* **45**, 204 (1941)
9 Bauman, L *Am J Med Sci* **200**, 299 (1940)
10 Bell, H J , Best, C H , and Haist, R E *J Physiol* **101**, 11 (1942)
11 Bérard and Colin *Gaz Hebdomadaire de méd et chir* **5**, 59 (1858)
12 Bernard, Claude *Leçons de Physiol* II, 274 (1856)
13 Best, C H , Campbell, J , Haist, R E , and Ham, A W *J Physiol* **101**, 17 (1942)
14 Biasotti, A , Deulofeu, V , Mendive, J R , and Patalano, A *Medicina* **3**, 442 (1943)
14a Biskind, M S , and Schreier, H *Exptl Med Surg* **3**, 299 (1945)
15 Bliss, C I , and Marks, H P *Quart J Pharm Pharmacol* **12**, 82 (1939)
16 Bliss, C I , and Marks, H P *ibid* **12**, 182 (1939)
17 Bodo, R C , Co Tui, F , and Farber, L *Am J Physiol* **103**, 17 (1933)
18 Bodo, R C , and Neuwirth, I *ibid* **103**, 5 (1933)
19 Bouchardat, A De la glycosuria ou diabète sucré, son traitement hygyenique avec notes et documents sur la nature et le traitement de la goutte Germer-Baillière, Paris, 1883
20 Brand, E , Ryan, F J and Diskant, E M *J Am Chem Soc* **67**, 1532 (1945)

21. Bridge, E M *Bull Johns Hopkins Hosp* **62**, 408 (1938).
22 von Brunner, J C Experiments nova circum pancreas Accedit diatribe de lympha et genuine pancreatis usu Amsteloedami, apud H Wetstenium, 1683
23 Bueding, E , Fazekas, J F , Herrlich, H , and Himwich, H E *J Biol Chem* **148**, 97 (1943)
24 Bueding, E , and Goldfarb, W *ibid* **147**, 33 (1943)
25 Burn, J H Biological Standardization Oxford Medical Publications, London, 1937
26 Chesler, A , and Himwich, H E *J Biol Chem* **155**, 413 (1944)
27 Chevreul, M E *Ann chim* **95**, 319 (1815)
28 Chibnall, A C *Proc Roy Soc London* **B131**, 153 (1942)
28a Chibnall, A C *J Intern Soc Leather Trades' Chemists* **30**, 1 (1946)
29 Cohn, E J , Ferry, J D , Livingood, J J , and Blanchard, M H *J Am Chem Soc* **63**, 17 (1941)
30 Colowick, S P , and Sutherland, E W *J Biol Chem* **144**, 423 (1942)
31 Colwell, A R *Arch Internal Med* **74**, 331 (1944)
32 Conn, J W , and Louis, L *J Clin Endocrinol* **5**, 247 (1945)
33 Copp, E F F , and Barclay, A J *J Metabolic Research* **4**, 445 (1923)
34 Cori, C F *J Biol Chem* **70**, 577 (1926)
35 Cori, C F *Biol Symposia* **5**, 131 (1941)
36 Cori, C F , and Cori, G T *J Biol Chem* **94**, 581 (1931)
37 Crammer, J L , and Neuberger, A *Biochem J* **37**, 304 (1943)
38 Crowfoot, D *Chem Revs* **28**, 215 (1941)
39 Del Fierro, R S , and Sevringhaus, E L *Ann Internal Med* **22**, 667 (1945)
40 Diamare, V *Intern Monatschr Anat Physiol* **16**, 155 (1899)
41 Dobson, M *Med Observ and Inquiries* **5**, 259 (1776)
42 Dominicis, N de *Munch med Wochschr* **38**, 717 (1891)
43 Dorfman, A *Physiol Revs* **23**, 124 (1943)
44 Dragstedt, L R , Allen, J G , and Smith, E M *Proc Soc Exptl Biol Med* **54**, 292 (1943)
45 Dunn, J S , Duffy, E , Gilmour, M K , Kirkpatrick, J , and McLetchie, N G B *J Physiol* **103**, 233 (1944)
46 Dunn, J S , Kirkpatrick, J , McLetchie, N G B , and Telfer, S V *J Path Bact* **55**, 247 (1943)
47 Dunn, J S , and McLetchie, N G B *Lancet* **245**, 384 (1943)
48 Eaton, J C *ibid* **247**, 269 (1944)
49 Eisenbrand, J , Stenz, M , and Wegel, F *Chem Abstracts* **38**, 4666 (1944)
50 Fieller, E C , Irwin J O , Marks, H P , and Shrimpton, E A G *Quart J Pharm Pharmacol* **12**, 206 (1939)
51 Fieller, E C , Irwin, J O , Marks, H P , and Shrimpton, E A G *ibid* **12**, 724 (1939)
52 Fraenkel Conrat, H L *Ann Rev Biochem* **12**, 276 (1943)
53 Freudenberg K , and Münch, A *Z physiol Chem* **263**, 1 (1940)
54 Gaebler, O H , and Ciszewski, W E *Endocrinology* **36**, 227 (1945)
55 Gelling, E M K , Jensen, H , and Farrar, G E , Jr Insulin Handbuch der Experimentellen Pharmakologie, Ergaenzungswerk V 197, Springer, Berlin, 1937
56 Gellhorn, E , Feldman, J , and Allen, A *Endocrinology* **29**, 137 (1941)
57 Gellhorn, E Feldman, J , and Allen, A *ibid* **29**, 849 (1941)

58 Gemmill, C L *ibid* 68, 329 (1941)
59 Gemmill, C L, and Hamman, L, Jr *Bull Johns Hopkins Hosp* 68, 50 (1941)
60 Gley, E *Compt rend soc biol* 43, 752 (1891)
61 Gley, E, and Camus L *ibid* 53, 194 (1901)
62 Goldner, M G, and Clark, D F *J Clin Endocrinol* 4, 194 (1944)
63 Goldner, M G and Gomori G *Endocrinology* 33, 297 (1943)
63a Goldner, M G, and Gomori, G *ibid* 35, 241 (1944)
64 Goldner, M G, and Gomori, G *Proc Am Diabetic Assoc* 4, 87 (1944)
65 Gomori, G *Arch Path* 36, 217 (1943)
66 Gomori, G *Bull N Y Acad Med* 21, 99 (1945)
67 Hagedorn, H C, Jensen, B N, Krarup, N B, and Wodstrup, K *J Am Med Assoc* 106, 177 (1936)
68 Haist, R E *J Physiol* 98, 419 (1940)
69 Haist, R E *Physiol Revs* 24, 409 (1944)
70 Haist, R E, and Bell, H J *Am J Physiol* 141, 606 (1944)
71 Hall, J L *J Biol Chem* 139, 175 (1941)
71a Hallas-Moeller, K Chemical and Biological Insulin Studies, NYT Nordisk Forlag Arnold Busck, Copenhagen 1945
72 Harington, C R, and Neuberger, A *Biochem J* 30, 809 (1936)
73 Hechter, O, Levine, R, and Soskin, S *Proc Soc Exptl Biol Med* 46, 390 (1941)
74 Hédon, E *Compt rend soc biol* 44, 307 (1892)
75 Hédon, E *ibid* 44, 678 (1892)
76 Hill, D, and Howitt, F Insulin, its production purification and physiological action Hutchinson's Scientific and Technical Publication, London, 1936
77 Homans, J *J Med Research* 30, 49 (1914)
78 Houssay, B A *Endocrinology* 30, 884 (1942)
79 Houssay, B A *ibid* 35, 158 (1944)
80 Houssay, B A, and Deulofeu, V *Ergeb Vitamin- u Hormonforsch* 2, 297 (1939)
81 Houssay, B A, Foglia, V G, and Smyth, F S *J Exptl Med* 74, 283 (1941)
82 Hughes, H, Ware, L L, and Young F G *Lancet*, 246, 148 (1944)
83 Ingle, D J *Endocrinology* 29, 649 (1941)
84 Ingle, D J *ibid* 31, 419 (1942)
85 Ingle, D J The Chemistry and Physiology of Hormones, 83 American Association for the Advancement of Science, Washington, D C, 1944
86 Ingle, D J Sheppard, R, Evans, J S, and Kuizenga M H *Endocrinology* 37, 341 (1945)
87 Irwin, J O *Quart J Pharm Pharmacol* 16, 352 (1944)
88 Jensen, H Insulin, Its Chemistry and Physiology The Commonwealth Fund, New York, 1938
89 Jensen, H The Chemistry and Physiology of Hormones, 69 American Association for the Advancement of Science, Washington, D C, 1944
90 Jensen, H, and Casey, J J Unpublished
91 Jensen, H, and Evans, E A, Jr *Physiol Revs* 14, 183 (1934)
91a Jensen, H, and Evans E A, Jr *J Biol Chem* 108, 1 (1935)
92 Jensen, H, Wintersteiner, O, and Geiling, E M K *J Pharmacol* 36, 115 (1929)
93 Joslin, E P *New Eng J Med* 230, 425 (1944)
94 Kaplan, N O, and Greenberg, D M *J Biol Chem* 156, 525 (1944)

95 Kaplan, N O, and Greenberg, D M *Am J Physiol* **140**, 598 (1944)
96 Kaplan, N O, and Greenberg D M *J Biol Chem* **156**, 553 (1944)
97 Kauer, J T, and Glenn, F *Am J Physiol* **131**, 437 (1941)
98 Keenan, G L *Am Pharm Assoc* **33**, 183 (1944)
99 Kendall, E C *Arch Path* **32**, 474 (1941)
100 Kennedy, W B, and Lukens, F D W *Proc Soc Exptl Biol Med* **57**, 143 (1944)
101 Kenyon, A T, Knowlton, K, and Sandiford, I *Ann Internal Med* **20**, 632 (1944)
101a Keston, A S, Udenfriend, S, and Cannan, R K *J Am Chem Soc* **68**, 1390 (1946)
102 Klebs, E, and Munk, P Tageblatt der 43 Versammlung deutscher Naturforscher und Aerzte in Innsbruck, 1869
103 Krebs, H A *Advances in Enzymol* **3**, 191 (1943)
104 Krebs H A, and Eggleston, L V *Biochem J* **32**, 913 (1938)
105 Laguesse, M E *Compt rend soc biol* **45**, 819 (1893)
106 Laguesse, M E *Compt rend soc biol* **45**, 819 (1893)
107 Lang, E H, and Reiner, L *Science* **93**, 401 (1941)
108 Langerhans, P Beitrage zur mikroscopischen Anatomie der Bauchspeicheldruse Dissertation, Berlin, G Lange, 1869
109 Lawrence, R D *Brit Med J* **2**, 103 (1943)
109a Lens, J *J Biol Chem* **164**, 223 (1946)
110 Lépine, R Diabète pancréatique expérimental *Lyon méd* **74**, 415 (1893)
111 Lerman, J *Am J Med Sci* **207**, 354 (1944)
112 Lewis, J C, and Olcott, H S *J Biol Chem* **157**, 265 (1945)
113 Long C N H *Proc Am Diabetic Assoc* **2**, 99 (1942)
114 Long, C N H *Ann N Y Acad Sci* **43**, 383 (1943)
115 Lukens, F D W, and Dohan, F C *Endocrinology* **30**, 175 (1942)
116 Lukens, F D W *Yale J Biol Med* **16**, 301 (1944)
117 MacBryde, C M, and Reiss, R *J Clin Endocrinol* **4**, 469 (1944)
118 MacBryde, C M, and Roberts, H K *J Am Med Assoc* **122**, 1125 (1943)
119 MacBryde, C M and Roberts, H K *J Clin Invest* **22**, 791 (1943)
120 Macleod, J J R *J Metabolic Research* **2**, 149 (1922)
121 Martin, H E, Simonsen, D G, and Homan, N H *Am J Med Sci* **208**, 321 (1944)
122 von Mehring, J, and Minkowski, O *Arch exptl Path Pharmakol* **26**, 371 (1890)
123 de Meyer, J *Arch fisiol* **7**, 96 (1909)
124 Miller, G L, and Anderson, K J I *J Biol Chem* **144**, 459 (1942)
125 Miller, G L, and Anderson, K J I *ibid* **144**, 465 (1942)
126 Miller, G L, and Anderson, K J I *ibid* **144**, 475 (1942)
127 Minkowski O *Klin Wochschr* **190**, 90 (1892)
128 Minkowski O *Arch exptl Path Pharmakol* **31**, 85 (1892)
128a Mirsky, I A *Am J Digestive Diseases* **13**, 130 (1946)
129 Mirsky, I A, Nelson N, Elgart, S, and Grayman, I *Science* **95**, 583 (1942)
130 Mirsky, I A, Nelson N, Grayman, I, and Korenberg, M *Am J Physiol* **135**, 223 (1941)
131 Mosenthal, H O *J Am Med Assoc* **125**, 483 (1944)
132 Needham, J Chemical Embryology Cambridge Univ Press, London, 1931
133 Nelson, N, Elgart, S, and Mirsky, I A *Endocrinology* **31**, 119 (1942)

134 Nicolet, B H, and Shinn, L A *J Am Chem Soc* **63**, 1486 (1941)
135 Olcott, H S *J Biol Chem* **153**, 71 (1944)
136 Opdyke, D F *Am J Physiol* **139**, 563 (1943)
137 Pauls, F, and Drury, D R *J Biol Chem* **145**, 481 (1942)
138 Peck, F B, and Schechter, J S *Proc Am Diabetic Assoc* **4**, 59 (1944)
139 Price, W H, Cori, C F, and Colowick, S P *J Biol Chem* **160**, 633 (1945)
140 Reiner, L, Keston, A S, and Green, M *Science* **96**, 362 (1942)
141 Reiner, L, and Lang, E H *J Biol Chem* **139**, 641 (1941)
142 Reiner, L, Lang, E H, Irvine, J W, Jr, Peacock, W, and Evans, R D *J Pharmacol* **78**, 352 (1943)
143 Rice, L, and Evans, E A, Jr *Science* **97**, 470 (1943)
144 Richardson, K C, and Young, F G *J Physiol* **91**, 352 (1937)
145 Ricketts, H T *Illinois Med J* **87**, 133 (1945)
146 Ricketts, H T, Brunschwig, A, and Knowlton, K *Proc Soc Exptl Biol Med* **58**, 254 (1945)
147 Roberts, S, and Samuels, L T *ibid* **53**, 207 (1943)
148 Romans R G, Scott D A, and Fisher, A M *Ind Eng Chem* **32**, 908 (1940)
149 Root, H F, Irvine, J W, Jr, Evans, R D, Reiner, L, and Carpenter, T M *J Am Med Assoc* **124**, 84 (1944)
150 Russell, J A *Ann Rev Biochem* **14**, 322 (1945)
151 Ryan, F J, and Brand, E *J Biol Chem* **154**, 161 (1944)
152 Sacks, J *Am J Physiol* **143**, 157 (1945)
153 Sahyun, M *J Biol Chem* **138**, 487 (1941)
153a Sanger, F *Biochem J* **39**, 507 (1945)
154 Schiff, M *Centr Med Wissen* **10**, 790 (1872)
155 Schulze, W *Arch mikroskop Anat (Bonn)* **56**, 491 (1900)
156 Scott, D A *J Biol Chem* **92**, 281 (1931)
157 Scott, D A *Endocrinology* **25**, 437 (1939)
158 Scott, D A, and Fisher, A M *J Pharmacol* **58**, 78 (1936)
159 Scott, D A, and Fisher, A M *Trans Roy Soc Can* **34**, 137 (1940)
160 Scott, D A, and Fisher, A M *ibid* **36**, 45 (1942)
161 Sharpey-Schafer, E The Endocrine Organs 2nd ed, Part 2, Chap 49 Longmans, Green, New York, 1926
162 Smith, K W, Marks, H P, Fieller, E C, and Broom, W A *Quart J Pharm Pharmacol* **17**, 108 (1944)
163 Soskin, S *Arch Internal Med* **71**, 219 (1943)
164 Soskin, S *J Clin Endocrinol* **4**, 75 (1944)
165 Soskin, S, and Levine, R *Am J Physiol* **129**, 782 (1940)
165a Soskin, S, and Levine, R Carbohydrate Metabolism Univ Chicago Press, Chicago, 1946
166 Soskin, S, Levine, R, and Hechter, O *Am J Physiol* **134**, 40 (1941)
167 Ssobolew, L W *Arch path Anat Physiol Virchow's* **168**, 91 (1902)
168 Stadie, W C *Harvey Lectures* **37**, 129 (1942)
169 Stadie, W C *Yale J Biol Med* **16**, 539 (1944)
169a Stadie, W C, Lukens, F D W, and Lapp, J A, Jr *J Biol Chem* **132**, 303 (1940)
170 Stetten, D, Jr, and Klein, B V *ibid* **159**, 593 (1945)
171 Susman, W *J Clin Endocrinol* **2**, 97 (1942)
172 Trasoff, A, Borden, C L, and Mintl, S S *Am J Digestive Diseases* **12**, 313 (1945)

173 du Vigneaud, V *Cold Spring Harbor Symposia Quant Biol* **6**, 275 (1938)
174 du Vigneaud, V, Brown, G B, and Bonsnes, R W *J Biol Chem* **141**, 707 (1941)
175 du Vigneaud, V, Miller, G L, and Rodden, C J *ibid* **131**, 631 (1939)
176 Warren, S The Pathology of Diabetes Mellitus Lea & Febiger, Philadelphia 1938
177 Warthin, A S, in Barker, L F Endocrinology and Metabolism, II, 725, Appleton, New York, 1922
178 Wasserman, P, and Mirsky, I A *Endocrinology* **31**, 115 (1942)
179 Weintraud, W *Arch exptl Path Pharmakol* **34**, 303 (1894)
180 Weissberger, L H *J Biol Chem* **160**, 481 (1945)
181 White A *Cold Spring Harbor Symposia Quant Biol* **6**, 262 (1938)
182 Willis T Pharmaceutics Rationalis Oxford, 1674
183 Wintersteiner, O, and Abramson, H A *J Biol Chem* **99**, 741 (1933)
184 Young, F G *Ann Rev Physiol* **6**, 434 (1944)
185 Young, F G *Practitioner* **154**, 129 (1945)
186 Young, F G *Brit Med J* **2**, 715 (1944)

Chapter IX

Assay of Ovarian Hormones

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Introduction

The numerous methods that have been used for the quantitative determination of biologically active substances may be divided into chemical (I), physical (II), and biological (III) Measurement of quantities and concentrations of the estrogens and of progesterone have been made by all three methods It is not possible to present all of the proposed techniques in detail Accordingly those which are practical for usual assay purposes will be explicitly presented and reference will be made to others where requisite In general assay methods have been employed for two purposes the determination of active hormones as chemicals or pharmaceuticals, and the determination of active hormones in body tissues and fluids In the former case macromethods are often applicable and desirable, in the latter case micromethods are ordinarily mandatory because of the minute or at best rather small concentration of these hormones in the body and its excreta

International Standards

Two estrogenic substances have been set up as International Standards under League of Nations conferences (69,70) on hormone standardization Crystalline estrone was contributed to the estrone reference standard and the international unit was defined as the specific estrus-producing activity contained in 0 1 μg The international unit of estradiol benzoate is 0 1 μg of this compound The international unit of progesterone is the specific progestational activity contained in 1 mg of crystalline hormone

I. Chemical Methods of Assay

A Estrogens

The most obvious and ultimately the definitive method of chemical measurement of presumed estrogens is the weighing of the isolated crystalline compounds and their identification by melting points, melting points of derivatives, and other physical characteristics This involves methods of fractionation and purification given in the next chapter, which need not concern us here The gravimetric determination of estriol in late human pregnancy urine and of estrone in mare's pregnancy urine is practicable in view of the relatively large amounts obtainable and the possibility of separating these compounds out almost quantitatively by fairly simple fractionation and subsequent chromatography In all other instances in which analysis of animal estrogens has been sought, isolation is not practicable unless extremely large quantities of starting material are used and ordinarily rather complex extraction procedures employed In the practice of either clinical analyses or research investigations the most common microchemical method of determination employed is an estrogen color reaction

Kober (45) was the first to propose a color reaction for urinary estrogen, he observed that phenolsulfonic acid reacted with estrone and urinary extracts to give a pink color Since Kober's publication various investigators have studied one or more modifications of his reaction and examined other colorimetric reactions of estrogens The pertinent publications are summarized in Table I It should be pointed out that in most mammalian tissues and fluids the naturally occurring estrogens are estrone, estradiol (β-estradiol in the rabbit, α-estradiol in all other mammals examined, see Pincus and Pearlman, 59, and Doisy, 25), and estriol Equilin, equilenin, and related estrogens appear to be peculiar to the horse family Ideally one should have a specific color reaction for each of these that may be developed quantitatively in the presence of other compounds present in the extractions containing the estrogens

This ideal has not been met In every procedure proposed in Table I a greater or lesser amount of purification of the original sources of the estrogens is required In none of the purification procedures employed is an absolute quantitative yield of all of the estrogens assured Furthermore in most instances accompanying estrogens in the final extracts are nonestrogenic chromogens which react with the reagents employed to give either the same or some other color It appears probable that the urines of late pregnancy may be assayed colorimetrically for their estrogen content By all methods, except possibly that of Stimmel, the estrogen content of nonpregnancy or early pregnancy urines is not ascertainable due to the presence of interfering chromogens Even in Stimmel's method the use of a color correction equation is requisite (78), and then can apparently be applied safely only to the midmenstrual peak of excretion in women Finally none of the methods appear to be applicable to extracts of blood body tissues feces, or the urines of men

B Progesterone

The gravimetric determination of pure progesterone as isolated from corpus luteum tissue or whole ovaries is the only quantitative method known No practicable color reactions have been proposed The almost complete absence of progesterone from urine has not encouraged the sort of development observed in estrogen microcolorimetry

The chief metabolite of progesterone is pregnanediol It has been measured quantitatively in pregnancy urine and in the urines of the luteal phase of the menstrual cycle as pregnanediol glucuronide by the method of Venning (87) "Spontaneous" hydrolysis of the glucuronide must be guarded against by preserving the urine with sodium cyanide Colorimetric measurement of the glucuronic acid freed on hydrolysis has been advocated to avoid certain steps in the Venning procedure (42) Where sufficient quantities of pregnanediol are present, direct isolation is advocated (Astwood and Jones 5) The method of Astwood and Jones gives values 20% to 40% lower than those obtained with the Venning method (Bachman, 8) Marrian's recent finding (51) that glucuronides obtained by the Venning method contain considerable amounts of the pregnanolones may account for this difference A color reaction of pregnanediol with sulfuric acid (82) can be applied only if the free pregnanediol is relatively uncontaminated by impurities Its application to the neutral alcoholic, nonketonic fraction of human urine has therefore been suggested (56)

The pregnanolones are 20 ketosteroids found in human pregnancy urines Although chemically quite similar to pregnanediol they prob-

TABLE 1

COLORIMETRIC METHODS FOR THE ASSAY OF ESTROGENS

Authors	Ref no	Compounds used	Fluids or tissues used	Color reaction	Extraction methods	Remarks
Cohen and Marrian	16	Estrone Estriol	Human urine	Heat with phenolsulfonic acid, cool in ice bath and dilute with 5% H_2SO_4, cool and measure pink color	Acid hydrolysis, separation of "weak" phenols from "strong" phenols	Applicable only to late pregnancy urines, non-estrogenic chromogen contribution probable, estrone and estradiol in same fraction
David	23	Estriol	None	Heat with concentrated H_2SO_4, dilute and add arsenic acid, measure blue color	None	Specific for estriol
Sola	76	Estrone	Mare's pregnancy urine	Heat with concentrated H_2SO_4, and measure greenish fluorescence	Acid hydrolysis, separation of urinary phenols	Qualitative test only
Zimmerman	93	Estrone	None	Add alcoholic *m*-dinitrobenzene and concentrated KOH solution, and measure pink color	None	All ketones react with this reagent but 17-ketones give characteristic pink color
Cartland, Meyer, Miller, and Rutz	13	Estrone	Stallion urine	Heat with phenolsulfonic and cool, dilute with H_2O, heat, cool, dilute with H_2O, and measure pink color	Butanol extraction, acids removed, and Butenandt (11) partition followed	"Cloudy" solutions often develop with crude urine extracts
Schmulovitz and Wylie	74	Estrone (theelin)	Human pregnancy urine	Diazotized *p*-nitroaniline added to alcoholic $NaCO_3$ solution, "wine" color measured	Urine concentrated at pH 4, extracted with ethyl ether, washed with $NaCO_3$, ether and volatile phenols distilled off	Used for pregnancy diagnosis

Pincus, Wheeler, Young, and Zahl	63	Estrone Estradiol Estriol	Human urine, rabbit urine	(*a*) Cohen and Marrian procedures, (*b*) procedures of Cartland *et al*, (*c*) David procedure (*d*) to hormone in chloroform solution add $ZnCl_2$ solution followed by benzoyl chloride, heat, cool, dilute with chloroform, and measure color	Acid hydrolysis, separation of "weak" phenols from "strong" phenols	(*a*) Applicable only to late pregnancy urines, better than (*b*) for these; both react with nonestrogenic chromogens in rabbit urines (*c*) specific but not applicable to urine extracts as purified (*d*) estriol does not react; checks with (*a*) in late pregnancy urines, gives intense reaction with nonestrogenic chromogens in rabbit urines
Pincus and Zahl	64	Estrone Estradiol Estriol	Rabbit urine	Sulfanilic acid and $NaNO_2$ in aqueous solution added to alcoholic hormone solution, coupling on adding NaOH solution, measure red color	Acid hydrolysis, separation of "weak" phenols from "strong" phenols	Applicable only to unpigmented extracts, less sensitive than Kober reaction, estrone and estradiol in same fraction
Venning, Evelyn, Harkness, and Browne	89	Estrone Estradiol Estriol	Human urine	Phenolsulfonic acid added to dry extract, heated 20 min, diluted with H_2O after cooling, reheated, cooled and 10% H_2SO_4 added Pink color developed	Butanol extraction of acidified urine, acid hydrolysis of butanol extract and ether extract of hydrolyzate	Applicable to human pregnancy urines containing 500 μg or more of estrogen, color correction for nonestrogenic chromogen necessary, some inhibition of color development by pregnanediol

TABLE I (*Continued*)

Authors	Ref no	Compounds used	Fluids or tissues used	Color reaction	Extraction methods	Remarks
Bachman, Bachman, and Pettit	6 7,9	Estrone Estradiol Estriol	Human urine	(*a*) Cohen and Marrian procedure applied to estrone estradiol mixture (*b*) Zimmerman reaction applied to estrone estradiol mixture, (*c*) sodium *p* phenolsulfonate in 85% H_3PO_4 heated at 150°, cooled in ice, and violet-pink color measured with estriol	Acid hydrolysis, separation of estriol by 0 9 *M* Na_2CO_3 out of benzene and segregation of estrone and estradiol into a second fraction	Applicable only to pregnancy urines from 35th week to term
Talbot, Wolfe, MacLachlan, Karusch and Butler	83	Estrone	Human urine	2% Na_2CO_3 added to absolute ethanol solution of ketones followed by diazotized dianisidine and toluene red color	Acid hydrolysis, "strong" phenols discarded, "weak" phenols after NaOH hydrosulfite wash subjected to micro-Girard, and ketones used	Applicable to pregnancy urines and other female urines, 20–40% loss of estrone in extraction; no bioassay check
Szego and Samuels	81	Estrone Estradiol Estriol	Cow endometrium	Concentrated H_2SO_4 added to absolute ethanol solution of hormones in cold, heated, cooled, and potassium guaiacol sulfonate added, heated, diluted with 50% H_2SO_4 after standing, and pink color measured	Acid hydrolysis, chloroform extraction, pentane, ethanol distribution, adsorption out of acetone-pentane mixture, ketones separated by micro-Girard	Applicable only to estrogens incubated with endometrium, estriol measured slightly, declared not applicable to urine extracts (79); losses of 20–58% of estrogen in extraction procedure

Jayle, Crepy, and Judas	41	Estrone	Human urine	Kober reaction with acetone added, which preserves nonestrogenic colors overnight while pink color fades	Acid hydrolysis, ether extraction, "estriol" taken out of benzene solution of ether extract and estrone-estradiol mixture left	Applicable to human pregnancy urine containing 500 μg or more of estrogen Find enzymic hydrolysis of estrogens useless
Reifenstein and Dempsey	68	Estrone	Human urine	Procedure of Talbot *et al*	Acid hydrolysis, "strong" phenol removed, "weak" phenols subjected to micro-Girard separation and ketone fraction assayed	*Apparently overestimates* estrone due to nonestrogenic chromogens, 27–54% of estrone lost in extraction
Veitch and Milone	86	Estrone	None	2,4 dinitrophenylhydrazone of estrone added to 0 1 *N* alcoholic KOH and deep red color measured	Chromatography of hydrazone suggested	Applicability to tissues or fluids not known
Stimmel	77 78	Estrone Estradiol Estriol	Human urine	Kober reaction to "estradiol" fraction, Zimmerman (93) reaction to estrone fraction and Bachman (9) reaction to estriol fraction	Butanol extract acid hydrolyzed, acids removed with carbonate and chromatography of ether concentrate out of benzene	Applicable to pregnancy urines from 24th week on and to midmenstrual rise with color correction

ably do not represent metabolites of progesterone (59), but are more likely metabolites of adrenal steroid precursors (88)

II Physical Methods of Assay

A Estrogens—Absorption Spectrophotometry

The natural estrogens show a characteristic absorption spectrum in the ultraviolet due to the presence of the phenolic ring The absorption maximum is at 280 mμ in ethanol solution The equilenin series differs from the estrone series because of the naphthalenic structure of the former (43) Chevallier *et al* (14) have used this absorption peak as a qualitative test for estrone in mare's pregnancy urine, but in most urines there are interfering substances in crude fractions Friedgood and Garst (32) have recently re-examined the problem of the conditions necessary for the separation of mixtures of pure estrone, estradiol and estriol by methods which entail minimal losses and typical ultraviolet absorption curves A procedure involving 48 extraction steps was evolved, but it has not been applied to biological specimens

The infrared spectrum of the estrogens has been examined with a view to their assay by this method (33) The estrogens are distinguished by four bands at 11 30 to 11 42, 8 60 to 8 70, 6 20 to 6 30, and 6 65 to 6 70μ Thus far no application to biological specimens has been reported

B Progesterone—Absorption Spectrophotometry

The conjugated ketone of ring A of progesterone shows a typical ultraviolet absorption maximum at 240 mμ (in ethanol) This type of absorption is exhibited by the other steroid hormones having this $\alpha\beta$ unsaturated ketone structure (see 43) Reynolds and Ginsburg (71) have made an approach to its utilization as a means of detecting progesterone in blood Its practical application has, however, not been realized as yet The conjugated 3-carbonyl in steroids is associated also with an absorption maximum in the infrared at 1678 cm^{-1} (43), but specific recognition of progesterone in the presence of other absorbing compounds has not been achieved

C Estrogens—Polarographic Assay

Estrone couples with the ketone reagent of Girard to give a water-soluble compound having a specific current discharge on reduction at the dropping mercury electrode measurable polarographically (92) By appropriate adjustment microquantities of this ketone complex may be measured (10 90) The neutral 17 ketosteroids of human urine have been measured by this method (90,92), and the measurement of estrone is also being attempted (91)

III Biological Assay Methods

A Estrogens—Vaginal-Smear Methods

Standard estrogen bioassays are based on the Allen-Doisy vaginal-smear method (2,44) It has been modified considerably by numerous investigators in order to increase its sensitivity and reliability In each instance the essential procedure is the administration of standard estrogen to ovariectomized rats or mice and the examination of the vaginal smear at a given interval or various intervals after estrogen administration for the cornified epithelial cells that arise as the result of estrogen action in the otherwise quiescent vaginal epithelium On the basis of the percentage of animals showing cornified (positive) smears with increasing estrogen dosage a dosage–response curve is constructed Ordinarily the dosage which results in positive smears in 50% of the animals is designated as the unit of estrogen A considerable number of factors will determine the amount of estrogen necessary for a unit dose The role of various of these factors has been reviewed in a number of critical publications (18,22,24,29,44,52 55,67) Tables II and III illustrate most of the contributing variables

Table II is taken from the data of Pedersen Bjergaard (55) It demonstrates that identical methods of administration to different species lead to quite different assay units and that the ratio of activities between two estrogens varies from species to species The solvent employed and the frequency and route of administration all affect the derived assay unit Although taking the activity per weight of animal tends to reduce the marked difference in unit size from species to species (*e g* , the subcutaneous oily injections), wide variations are still encountered

Table III is taken from a paper by Thayer, Doisy, and Doisy (84) In testing the comparative activities of estrone β and α-estradiol and β-estradiol benzoate, five bioassay methods were employed These were as follows

1 *Modified Butenandt Procedure*

Single Injection of Estrogen in Oil, Mice (12) "Mice were ovariectomized as previously described by Allen and Doisy (1) Vaginal smears were made daily for a period of two weeks following ovariectomy and animals that did not show a negative smear each day were discarded Two weeks after ovariectomy, the mice were primed with an aqueous solution of estrone containing 0 1 γ or one international unit Smears were taken at 9 00 a m , 1 00 p m , and 5 00 p m , starting 48 hours after the injection

TABLE II

EFFECT OF METHODS OF ADMINISTRATION AND SPECIES OF TEST OVARIECTOMIZED ANIMALS ON THE ESTROGENIC BIOASSAY OF THE TWO INTERNATIONAL STANDARD PREPARATIONS

Animal	Solvent	Route[a]	No of administrations	Estrone		Estradiol benzoate		Relative potency, unit of estrone/unit of estradiol benzoate
				Assay unit, μg	Unit/20 g animal, μg	Assay unit, μg	Unit/20 g animal, μg	
Mouse	Oil	S	1	0 35	0 35	0 108	0 108	3 20
	Water	S	5	0 19	0.19	0 22	0 22	0 86
	Water	O	5	11 70	11 70	11 50	11 50	1 00
Rat	Oil	S	1	2 76	0 28	0 28	0 03	9 90
	Water	S	5	1 11	0 11	0 28	0 03	4 00
	Water	O	5	220 00	22 00	225 00	22 50	0 98
Guinea pig	Oil	S	1	600 00	15 00	45 00	1 13	13 00
	Water	S	5	460 00	11 50	30 00	0 75	15 00
	Water	O	5	8900 00	223 00	[b]		
Monkey	Oil	S	1	1000 00	5 00	25 00	0 13	40 00
	Water	O	1	5000 00	25 00	100,000 00	500 00	0 05

[a] S = subcutaneous, O = oral

[b] Ineffective at any level tested

TABLE III

POTENCIES OF β-ESTRADIOL, β-ESTRADIOL MONOBENZOATE, α-ESTRADIOL, AND ESTRONE AS DETERMINED BY FIVE DIFFERENT METHODS OF BIOASSAY

Compounds[a]	Butenandt modified units/mg mice	Marrian-Parkes modified units/mg mice	Thayer-Doisy units/mg mice	Allen-Doisy modified units/mg rats	Curtis-Doisy modified units/mg rats
β-Estradiol	700	8000	2000	200	700
α-Estradiol	35,000	70,000	40,000	17,000	5000
Estrone	20,000	35,000	20,000	1700	1000
β-Estradiol benzoate	1000	2400	450	200	200
R^1	28	4 4	10	8 5	1 4
R^2	1 4	0 3	0 22	1	0 3

[a] $R^1 = \frac{\text{Potency of estrone}}{\text{Potency of } \beta\text{-estradiol}}$, $R^2 = \frac{\text{Potency of } \beta \text{ estradiol-3-benzoate}}{\text{Potency of } \beta\text{-estradiol}}$

"One week after priming the mice were divided into two groups of 20 animals Each of one group of 20 animals was injected subcutaneously at 9 00 a m with the oily preparation being tested, each of a similar group was injected with the standard estrone preparation dissolved in oil, and the proportion of positive effects in each group was determined After one or two trial experiments, the dosage could usually be so adjusted that approximately the same percentage responses to the unknown and standard were obtained In accordance with the work of Coward and Burns (18) a 50 per cent positive response was regarded as one unit, the unknown was evaluated in terms of the standard response curve and the concurrent response to the standard

"Mice that failed to respond positively to an injection within 80 hours were immediately primed with an oily solution containing 0 05 γ of estrone Owing to the slow rate of absorption mice were not used again until four weeks had elapsed following a positive response The volume of oil injected was usually 0 2 cc and in all experiments the unknown and standard were administered in the same volume"

2 *Mather Modification of the Marrian-Parkes Procedure*

Four Injections of an Aqueous Solution, Mice (*a2,5S*) "Ovariectomized mice were given subcutaneous injections of four equal quantities of an aqueous solution at 8 00 a m and 5 00 p m , starting 16 hours after the last injection, and the last smear was taken 48 hours after the first smear In this method the same principles were followed as in the modified Butenandt method, using comparable animals and the same method of determining potency of a preparation The estrogens and the standard preparation used in this assay procedure were dissolved in an aqueous medium Animals that showed a negative response from previous injections were primed with an aqueous solution containing 0 1 γ of estrone before they were used for another assay In this method the test animals were used every two weeks"

3 *Thayer-Doisy Procedure*

Three Injections of an Aqueous Solution, Mice "Injections were made at 9 00 a m , 1 00 p m , and 5 00 p m on the same day The same principles regarding priming, estimation of activity, etc which were used in the preceding method were followed in this procedure, with the exception that the mice were used for assay at intervals of seven days We have used this method for many years as a routine procedure to determine the biological activity of estrogens"

4 *Allen-Doisy Procedure*

Three Injections of an Aqueous Solution, Rats (1) "Ovariectomized rats were given subcutaneous injections of three equal quantities of an aqueous solution at intervals of four and one-half hours All rats used had given a positive response one week before use for assay due to priming with 1 2 γ of estrone or a previous assay The same principles were followed as in the other procedures for determination of units In this method the changes of the cells in the vagina may be regarded as positive if a few leukocytes are present along with the nucleated epithelial cells and squamous nonnucleated epithelial cells "

5 *Curtis-Doisy Procedure*

Six Injections of Aqueous Solution, Immature Rats (19) "The estrogen was injected at 9 00 a m and 5 00 p m on three successive days Changes in the procedure from the original method as published by Curtis and Doisy were the requirement of opening of the vagina with a positive smear, and the application of the same procedure for determination of units as in the other assay methods "

These represent current and frequently employed methods of bioassay It may be seen that the latent period between ovariectomy, priming, and successive assays give marked differences in sensitivity (see methods 1, 2, and 3) as does the criterion of a positive response It should be noted that β-estradiol benzoate does not have the same potency as the free estrogen so that not only are there differences between the different estrogens but between derivatives of the same estrogen

There are other variables to be considered These are (*a*) the diet of the animals *e g*, vitamin A deficiency leads to vaginal cornification (31) and certain B complex deficiencies to absence of estrogen effect (35), (*b*) the method of taking vaginal smears (*e g*, too frequent smearing *per se* leads to vaginal cornification, 44), (*c*) seasonal or climatic changes that lead to significant fluctuations in the response of test animals (21,29 54) and (*d*) the peculiar insensitivity to estriol that develops on its repeated administration to the same animal (20)

It is clear from the foregoing that standard preparations of pure estrogen should be characterized by the name and specific physical and chemical constants of the contained substance If biological units are stated the exact method of assay should be given The *United States Pharmacopeia* in setting up reference standards for the individual estrogens is attempting to formulate a useful standard bioassay procedure An approved method, based on the use of spayed animals, should appear in the thirteenth edition of the *U S P*

B Estrogens—Other Methods

Other types of test animals and criteria of activity have been suggested as useful in estrogen assay The increase in uterine weight effected by estrogens in immature animals offers the opportunity for a graded response method Astwood (3) has suggested utilizing the increase in uterine water observed in rats at about six hours after estradiol administration It has the advantage of great sensitivity but the response to various estrogens is quantitatively different (62) The uterine hypertrophy occurring after several days administration may also be standardized (26 27,46), but an extensive characterization of controlling conditions has not been presented Extreme sensitivity is encountered when estrogen is applied intravaginally in castrated females (30,48,65), but again data sufficiently exact for standard conditions or quantification are lacking Recently an extremely sensitive response to estrogen, the disappearance of the vaginal closure membrane of the guinea pig, has been described (34) As little as 0 0008 μg of estradiol dipropionate gives a positive response, great variability, however, seems to characterize the effect (47)

In dealing with the bioassay of impure preparations a further complication is added to the variables encountered in the assay of crystalline estrogens In urine extracts for example, there occur unknown substances that may either augment or inhibit the activity of the contained estrogens (28,57) For such preparations, therefore, even an approximately accurate estimation of contained estrogens may be had only by purification and separation of fractions containing each individual estrogen Methods for such fractionation on a micro scale have been developed by several investigators (56–58,75), but they have been applied chiefly to urine Applicability to estrogen enriched serum perfusates has been indicated (72,73) The recent finding that blood estrogens are bound to protein (80) indicates the need for further refinement of methods in dealing with the naturally occurring compounds in this fluid and probably also in various organs and tissues

If bioassay of estrogens is to be practised with quantitative accuracy and reliability, it is clear that certain desiderata must be met The test animal should respond with minimum variability to the standard estrogen in easily repeatable fashion, the preparation to be tested must be sufficiently pure to represent the specific standard estrogen for which assay is sought The bioassay procedure for estrone in spayed rats presented by Curtis *et al* (21) appears to be very nearly the one of choice for estrone, a unit of 1 1 μg of estrone is repetitively encountered with an accuracy of $\pm 15\%$ (99 times out of 100) using fifteen to twenty animals

for each of three dosage levels Similar accuracy by a roughly similar method is obtained with estradiol and estriol (57) For greater sensitivity a completely reliable method is yet to be developed In any method employed the limits of accuracy must be strictly defined The necessary statistical methods have been excellently presented by Emmens (29), Curtis *et al* (21), and Pugsley (66) The bald statement of so many rat units or mouse units in a preparation is inadequate unless the limits of variation, the specific test, and the specific estrogen are included

C Progesterone

The progestational proliferation of the uterine endometrium of the rabbit in response to progesterone administration has been the basis of the chief methods of bioassay for this hormone The original method of Corner and Allen (17) requires the use of adult female rabbits ovariectomized at eighteen hours after a fertile mating A sample portion of the uterine horn removed at ovariectomy is sectioned and compared microscopically with a comparable portion removed one day after the last of five daily subcutaneous injections of progesterone in oil The degree of pseudopregnant proliferation in the latter sections is graded on a scale extending from − to ++++ The assay unit is set at a level given by +++, and in numerous assays this response has been obtained generally with one milligram of crystalline progesterone Increased sensitivity is had by the use of immature female rabbits primed with estrogen and then injected with progesterone (15,50), so that a unit dose is then about 0 6 to 0 7 mg The presence of estrogen in extracts to be assayed may vitiate these tests, and no statistical analysis setting the limits of accuracy is available

Pincus and Werthessen (60) have devised methods of measuring the endometrial response in mature female rabbits and have included measurements of the ovum growth response to progesterone A dosage of 0 38 mg progesterone may be detected in a single animal (98 times out of 100) Absolute accuracies may also be given on the basis of the number of animals employed for assay

The intrauterine application of progesterone to the rabbit permits the detection of small amounts of progesterone McGinty *et al* (49) were able to detect an effect of 0 13 μg in the primed immature rabbit Haskins (40) extended their observations and attempted quantification on the basis of the uterine mitoses resulting from progesterone stimulation Although he could measure 0 06 to 1 7 μg by this method, the variability was considerable The intrauterine method has been used to measure blood progesterone by Hoffmann and von Lám (37)

The contraction after adrenaline administration of the uterus of the

progesterone-treated cat is the basis for an assay proposed by Van Dyke and Chen (85) A good dosage-response curve is obtained with a unit of about 0 45 mg (the dosage per cat necessary to produce the uterine contraction in 50% of the animals)

Astwood (4) obtains a similar unit in measuring the minimum dosage required to elicit a deciduomatous response in the spayed pseudopregnant rat

The copulatory reflex in the spayed estrogen-sensitized guinea pig may be evoked by progesterone in small dosage Hertz, Meyer, and Spielman (36) have attempted to quantitate this response for assay purposes and obtain measurable results with as little as 0 05 mg per pig The variability has not been fully defined

The endometrial response of the rabbit is obtained also with desoxycorticosterone and methyltestosterone (39) It is not obtained with pregnanediol although pregnanediol may augment the activity of progesterone (61) Since these steroids and the estrogens may be easily excluded from progesterone preparations by ordinary extraction methods progesterone bioassay presents no special problem

When the detection of progesterone in biological specimens is sought all except the most sensitive methods are excluded due to the extremely low concentration of this hormone in most fluids and tissues Only corpora lutea, ovaries, and placentas have yielded easily quantifiable amounts Its excretion into urine does not normally occur, and it is reported that only 1/30,000th of administered hormone appears in the urine (38)

REFERENCES

1 Allen, E , and Doisy, E A *J Am Med Assoc* **81**, 819 (1923)
2 Allen, E , Doisy, E A , Francis, B F , Robertson, L L , Colgate, C E , Johnston, C G , Kountz, W E , and Gibson, H V *Am J Anat* **34**, 133 (1924)
3 Astwood E B *Endocrinology* **23**, 25 (1938)
4 Astwood, E B *J Endocrinology* **1**, 49 (1939)
5 Astwood, E B , and Jones G E S *J Biol Chem* **137**, 377 (1944)
6 Bachman, C *ibid* **131**, 455 (1939)
7 Bachman, C *ibid* **131**, 463 (1939)
8 Bachman, C *Am J Obstet Gynecol* **42**, 599 (1941)
9 Bachman, C , and Pettit, D S *J Biol Chem* **138**, 689 (1941)
10 Barnett, J , Henly, A A , and Morris, J O R. *Biochem J* **40**, 445 (1946)
11 Butenandt, A *Z physiol Chem* **191**, 127 (1930)
12 Butenandt, A , and Ziegner, E *ibid* **188**, 1 (1930)
13 Cartland, G F , Meyer, R K , Miller, L C , and Rutz, M H *J Biol Chem* **109**, 213 (1935)
14 Chevallier, A , Cornil, L., and Verdohn, J *Bull acad med* **114**, 171 (1935)
15 Clanberg, C *Zentr Gynäkol* **54**, 2757 (1930)
16 Cohen, S L., and Marrian, G F *Biochem J* **28**, 1603 (1934)

17 Corner, G W, and Allen, W M *Am J Physiol* **88**, 326 (1929)
18 Coward, K H, and Burns, J H *J Physiol* **63**, 270 (1927)
19 Curtis, J M, and Doisy, E A *J Biol Chem* **91**, 647 (1931)
20 Curtis, J M, Miller, L C, and Witt, E *ibid* **21**, 119 (1937)
21 Curtis, J M, Witt, E, and Knudsen, L E *Endocrinology* **34**, 149 (1944)
22 D'Amour, F E, and Gustavson, R G *J Pharmacol* **57**, 472 (1936)
23 David, K *Acta Brevia Néerland Physiol Pharmacol Microbiol* **4**, 64 (1934)
24 de Jongh, S E, Laqueur, E, and de Fremery, P *Biochem Z* **250**, 448 (1932)
25 Doisy, E A *Endocrinology* **30**, 933 (1942)
26 Dorfman, R I *Proc Soc Exptl Biol Med* **45**, 594 (1940)
27 Dorfman, R I, Gallagher, T F, and Koch, F C *Endocrinology* **19**, 33 (1935)
28 Emmens, C W *J Physiol* **94**, P2 (1938)
29 Emmens, C W *Med Research Council (Brit) Special Rept Series* **234** (1939)
30 Emmens, C W *J Endocrinol* **2**, 444 (1941)
31 Evans, H M, and Bishop, K S *Science* **56**, 650 (1922)
32 Friedgood, H B, and Garst, J Recent Progress in Hormone Research Vol II, p 31, Academic Press, New York, 1948
33 Furchgott, R F, Rosenkrantz, H, and Shorr, E *J Biol Chem* **163**, 375 (1946)
34 Hartman, C G, Littrell, J L, and Tom, J *Endocrinology* **39**, 120 (1946)
35 Hertz, R *ibid* **37**, 1 (1945)
36 Hertz, R, Meyer, R K, and Spielman, M A *ibid* **21**, 533 (1937)
37 Hoffmann, F, and von Lám, L *Zentr Gynäkol* **66**, 292 (1942)
38 Hoffmann, F, and von Lám, L *ibid* **67**, 1082 (1943)
39 Hohlweg, W *ibid* **63**, 1143 (1939)
40 Haskins, A L, Jr *Endocrinology* **27**, 983 (1940)
41 Jayle, M F, Crepy, O, and Judas, O *Bull soc chim biol* **25**, 301 (1943)
42 Jayle, M F, Crepy, O, and Wolf, P *ibid* **25**, 308 (1943)
43 Jones R N Recent Progress in Hormone Research Vol II, Academic Press, p 3, New York, 1947
44 Kahut, L C, and Doisy, E A *Endocrinology* **12**, 760 (1928)
45 Kober, S *Biochem Z* **239**, 209 (1931)
46 Lauson H D, Heller C G, Golden, J B, and Sevringhaus, E L *Endocrinology* **24**, 35 (1939)
47 Lloyd, C W, Rogers, W F, Jr, and Williams, R H *ibid* **39**, 256 (1946)
48 Lyons, W R, and Templeton, H J *Proc Soc Exptl Biol Med* **33**, 587 (1936)
49 McGinty, D A, Anderson, L P, and McCullough, N B *Endocrinology* **24**, 829 (1939)
50 McPhail, M K *J Physiol* **83**, 145 (1934)
51 Marrian, G F, and Gough, N *Biochem J* **40**, 376 (1946)
52 Marrian, G F, and Parkes A S *J Physiol* **67**, 27 (1929)
53 Mather, A *J Biol Chem* **144**, 617 (1942)
54 Palmer A *Univ Calif Pub Pharmacol* **1**, 375 (1941)
55 Pedersen Bjergaard, K Comparative Studies Concerning the Strengths of Estrogenic Substances Oxford Univ Press, London, 1939
56 Pincus, G *J Clin Endocrinol* **5**, 291 (1945)
57 Pincus, G, and Pearlman, W H *Cancer Research* **1**, 970 (1941)
58 Pincus, G, and Pearlman, W H *Endocrinology* **31**, 507 (1942)
59 Pincus, G, and Pearlman, W H *Vitamins and Hormones*, **1**, 294 (1943)
60 Pincus, G, and Werthessen, N T *Am J Physiol* **120**, 100 (1937)
61 Pincus, G, and Werthessen, N T *ibid* **124**, 484 (1938)

62 Pincus, G , and Werthessen, N T (Unpublished data)
63 Pincus, G , Wheeler, G , Young, G , and Zahl, P A *J Biol Chem* **116**, 253 (1936)
64 Pincus, G , and Zahl, P A *J Gen Physiol* **20**, 879 (1937)
65 Pratt, J P , and Smeltzer, M *Endocrinology* **13**, 320 (1929)
66 Pugsley, L I *ibid* **39**, 161 (1946)
67 Pugsley, L I , and Morrell, C A *ibid* **33**, 48 (1943)
68 Reifenstein, E C , Jr , and Dempsey, E F *J Clin Endocrinol* **4**, 326 (1944)
69 Report of the conference on the standardization of sex hormones Quart Bull Health Organ League of Nations **4**, 121, 1935
70 Report of the second conference on the standardization of sex hormones Quart Bull Health Organ League of Nations **4**, 618, 1935
71 Reynolds, S R M , and Ginsburg, N *Endocrinology* **31**, 147 (1942)
72 Schiller, J *ibid* **36**, 7 (1945)
73 Schiller, J , and Pincus, G *Science* **98**, 410 (1943)
74 Schmulovitz, M J , and Wylie H B *J Lab Clin Med* **21**, 210 (1935)
75 Smith O W , Smith, G V S , and Schiller, S *Endocrinology* **25**, 509 (1939)
76 Sola, S L *Rev sud-americana endocrinol inmunol quimioterap* **18**, 325 (1935)
77 Stimmel, B F *J Biol Chem* **162**, 99 (1946)
78 Stimmel, B F *ibid* **165**, 73 (1946)
79 Szego, C M *Lancet* **62**, 423 (1942)
80 Szego, C M , and Roberts, S *Proc Soc Exptl Biol Med* **61**, 161 (1946)
81 Szego, C M , and Samuels, L T *J Biol Chem* **151**, 587 (1943)
82 Talbot, N B , Berman, R. A , MacLachlan, E A , and Wolfe, J K *J Clin Endocrinol* **1**, 668 (1942)
83 Talbot, N B , Wolfe, J K , MacLachlan E A Karusch, F , and Butler, A M *J Biol Chem* **134**, 319 (1940)
84 Thayer, S A , Doisy, E A , Jr , and Doisy, E A *Yale J Biol Med* **17**, 19 (1944)
85 Van Dyke, H B , and Chen, J S *Endocrinology* **25**, 337 (1939)
86 Veitch, F P , and Milone H S *J Biol Chem* **158**, 61 (1945)
87 Venning, E H *ibid* **119**, 437 (1937)
88 Venning, E H *Endocrinology* **39**, 203 (1946)
89 Venning, E H , Evelyn K A , Harkness E V , and Browne, J S L *J Biol Chem* **120**, 225 (1937)
90. Werthessen, N T , and Baker, C F *Endocrinology* **36**, 351 (1945)
91 Werthessen N T , and Dyme, H C (Personal communication)
92 Wolfe J K , Hershberg, E B , and Fieser, L F *J Biol Chem* **136**, 653 (1940)
93 Zimmerman, W *Z physiol Chem* **233**, 257 (1935)

Chapter X

The Chemistry and Metabolism of the Estrogens

By William H Pearlman

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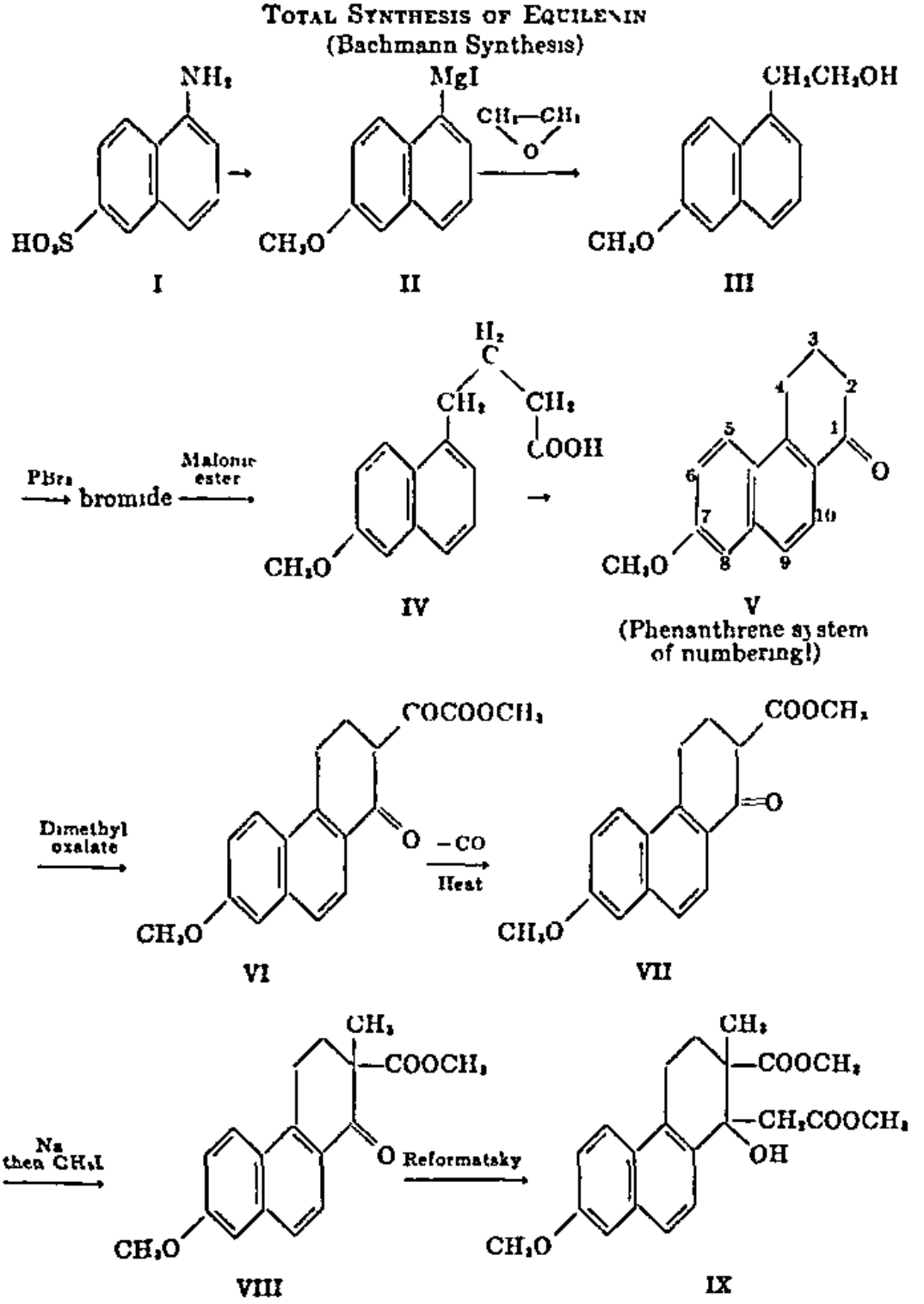
I Chemistry of Estrogens

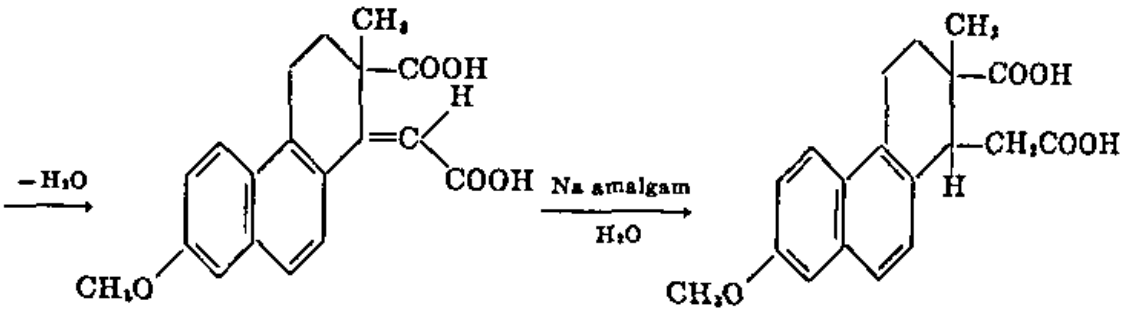
A Total Synthesis of Estrogens

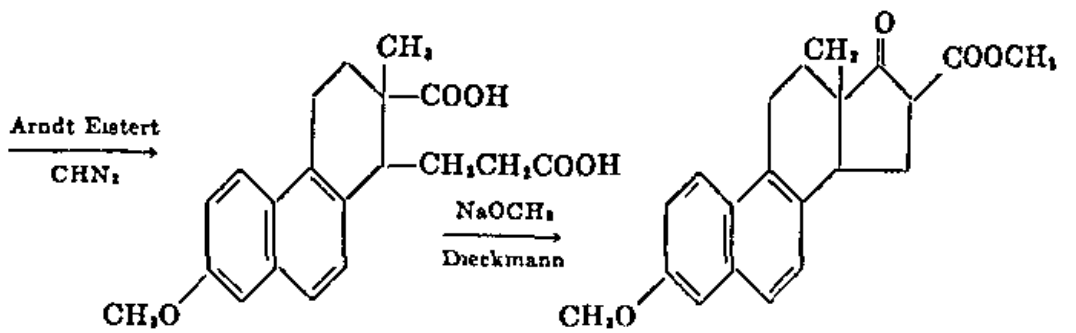
1 *Equilenin and Its Stereoisomers*

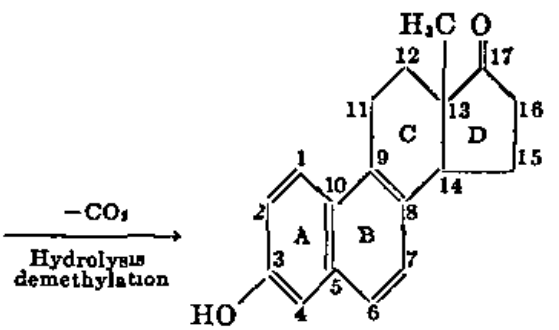
Of the many difficulties encountered in the path of total synthesis of the estrogens, the introduction of an angular methyl group between rings C and D was one of the most difficult to surmount In view of the many noteworthy attempts at total synthesis by other investigators (see the excellent review by Jones 96), the successful efforts of Bachmann and co-workers in 1939 and 1940 (3) are truly outstanding Starting with Cleve s acid, 1 aminonapthalene-6-sulfonic acid (I), equilenin (XIV) and its stereoisomers were obtained in 11 steps I was fused with sodium hydroxide to give aminonaphthol, diazotization and methylation yielded 6-methoxy 1 iodonaphthalene Its Grignard product (II) was treated with gaseous ethylene oxide to yield III, conversion to the corresponding bromide was effected by treatment with phosphorus tribromide The bromide was condensed with sodium malonic ester and the condensation product decarboxylated to give IV, which was converted to the acid chloride and cyclized to 1 keto 7 methoxy 1,2,3,4 tetrahydrophenanthrene (V) The latter compound was first prepared by Butenandt and Schramm in 1935 (30) from Cleve's acid but by a different route V was treated with dimethyl oxalate in the presence of sodium methoxide to yield the corresponding glyoxylate, VI Haworth had encountered difficulty in eliminating carbon monoxide from the glyoxalate but by the simple expedient of adding powdered glass and heating, Bachmann succeeded in obtaining VII The sodio derivative of VII reacted with methyl iodide whereupon an angular methyl group was introduced to give VIII Treatment with methyl bromoacetate in the Reformatsky

reaction yielded IX. Dehydration (in two steps) gave X. A geometric isomer of X was obtained as the acid anhydride and therefore the carboxyl groups may be assumed to be in *cis* relationship in this compound. The carboxyl group attached to the double bond in X extends







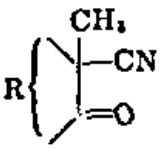


Equilenin or stereoisomers
(Steroid system of numbering!)

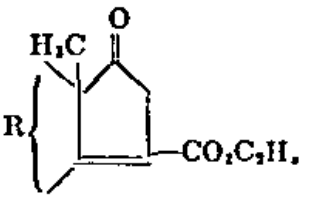
away from the tertiary carboxyl group Both acids (X) yielded on reduction with sodium amalgam in water the alpha and the beta forms of XI, which are actually racemic mixtures The arbitrary designation alpha and beta refers to the spatial configuration of the hydrogen atom on carbon atom 1 (phenanthrene system of numbering!) The beta acid subsequently yielded *dl*-equilenin, the alpha acid finally yielded *dl* isoequilenin (also independently synthesized recently by Birch *et al*, 12) This conversion (XI→XIV) was accomplished in the following way the beta acid was converted by the Arndt-Eistert method to the corresponding propionic acid derivative, XII Cyclization by the

Dieckmann method gave XIII, which on decarboxylation gave a racemic mixture of equilenin (XIV) The racemate was resolved by way of the *l*-menthoxyacetic ester to yield *d*-*n*-equilenin identical in every respect with the naturally occurring equilenin first isolated by Girard *et al* (62) from the urine of pregnant mares *d*-*Iso*-equilenin was found to be identical with the 14-*epi*-equilenin which had been prepared from equilenin by Hirschmann and Wintersteiner (81) The latter authors pointed out that the configuration of *d*-*iso*-equilenin at C_{13} is the same as that in naturally occurring equilenin Hence, the configuration of all stereoisomers of the natural hormone equilenin are established with reference to 14-*epi*-equilenin The estrogenic activity of *d*-*n*-equilenin is thirteen times that of *l*-*n*-equilenin, the *dl*-*iso*-equilenins are relatively inactive Since Marker (109) had previously succeeded in converting equilenin to estrone (XIX), the Bachmann synthesis of equilenin is in effect a synthesis of estrone

Johnson *et al* in 1945 (95) briefly described a new method for synthesizing equilenin Compound V (see above) was the starting point in the synthesis It was converted in three steps to



(R represents the nucleus of V) which reacted in a novel manner with diethyl succinate to yield



The latter substance was readily decarboxylated and then catalytically reduced to yield racemic mixtures of *n*- and *iso*-equilenin, these were resolved in the usual manner The new synthetic route may be useful in attempts to synthesize estrone

2 *Other Attempts at Total Synthesis*

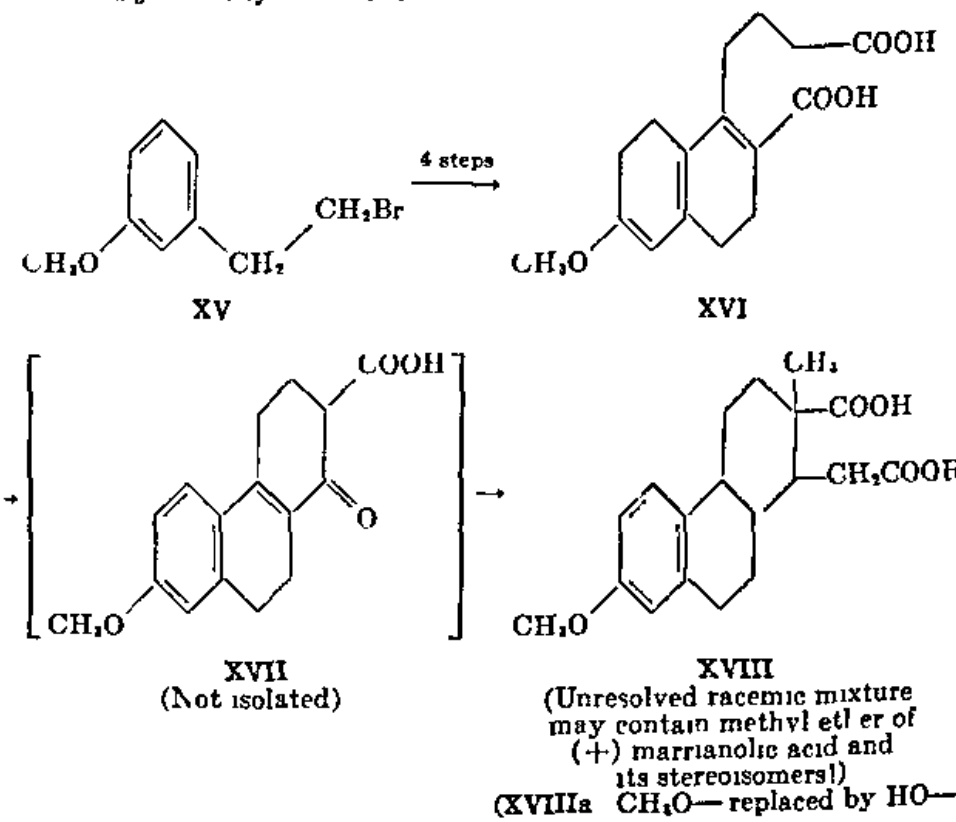
Bachmann and co-workers (6) applied the methods used in the equilenin synthesis in an attempt to synthesize estrone, this is a more difficult undertaking, since in estrone one has to reckon not only with the asymmetric centers at C-13 and C-14 as in equilenin but also with those at C-8 and C-9 The authors succeeded only in obtaining a stereoisomer of estrone It has been pointed out, however, that this method may some day give the desired result The synthesis was carried out as

follows the starting compound, β-*m*-anisylethyl bromide (XV) was condensed with sodiomalonic ester, and then in turn with the acid chloride of ethyl hydrogen glutarate The tricarboxylic ester formed was cyclized and partially decarboxylated to give XVI Treatment of the dimethyl ester of XVI with sodium methoxide gave the cyclic keto ester XVII, which was not isolated as such but used directly The conversion of XVII to *dl*-estrone a (XIX), was achieved in a manner essentially that used in the conversion of VII to XIV but the intermediate XVIII (compare with XI) was not separated into its stereoisomeric components *dl*-Estrone-a possesses only $\frac{1}{250}$ the activity of naturally occurring estrone It is significant that the resinous mixture from which *dl*-estrone a was obtained by direct crystallization is considerably more active

Dane and Schmidt (39) synthesized a stereoisomer or possibly an isomer of estrone 6-Methoxy-1-vinyl-3,4-dihydronaphthalene (XX) reacted with 1-methylpentene-2,3 dione (XXI) in a Diels Alder manner to give a 16,17 diketone (XXII) Reduction yielded the ketol XXIII, which was dehydrated and reduced to XIX The latter compound was not identical with estrone and the estrogenic activity was not reported

TOTAL SYNTHESIS OF ESTRONE ISOMERS

A Bachmann Synthesis of dl Estrone a

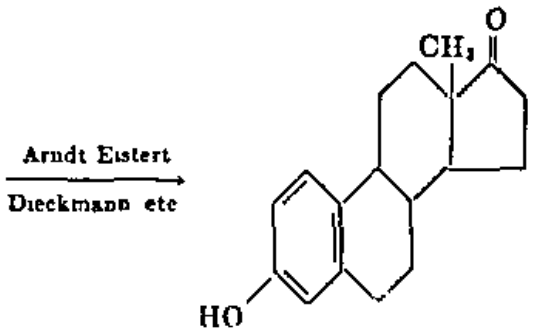


XV

XVI

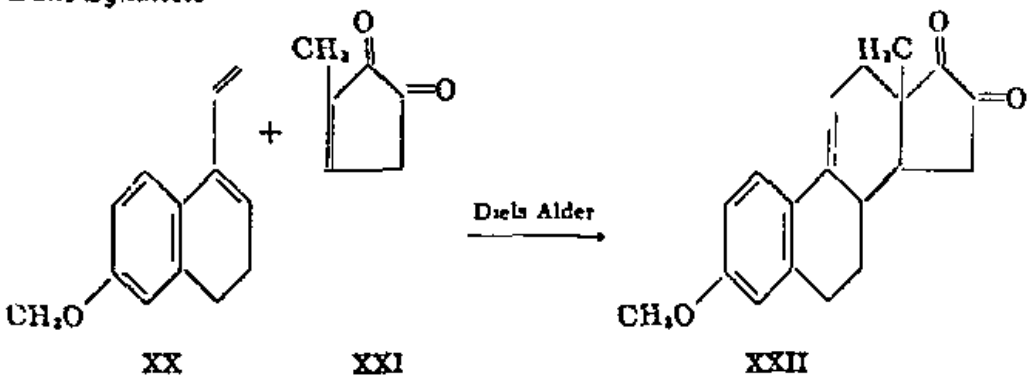
XVII
(Not isolated)

XVIII
(Unresolved racemic mixture may contain methyl ether of (+) marrianolic acid and its stereoisomers!)
(XVIIIa CH_3O— replaced by HO—)

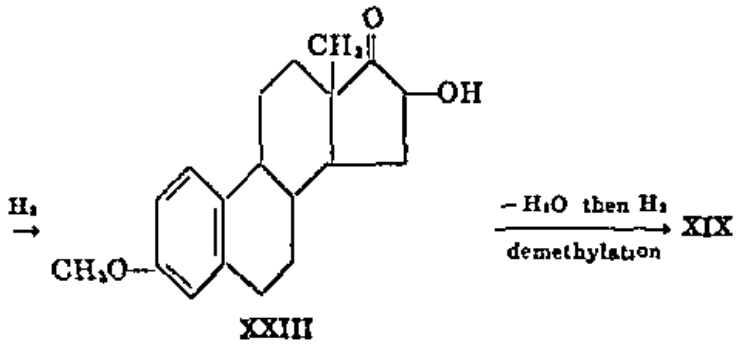


XIX
Estrone or stereoisomers

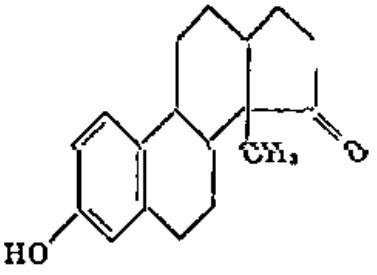
B Dane Synthesis



XX XXI XXII

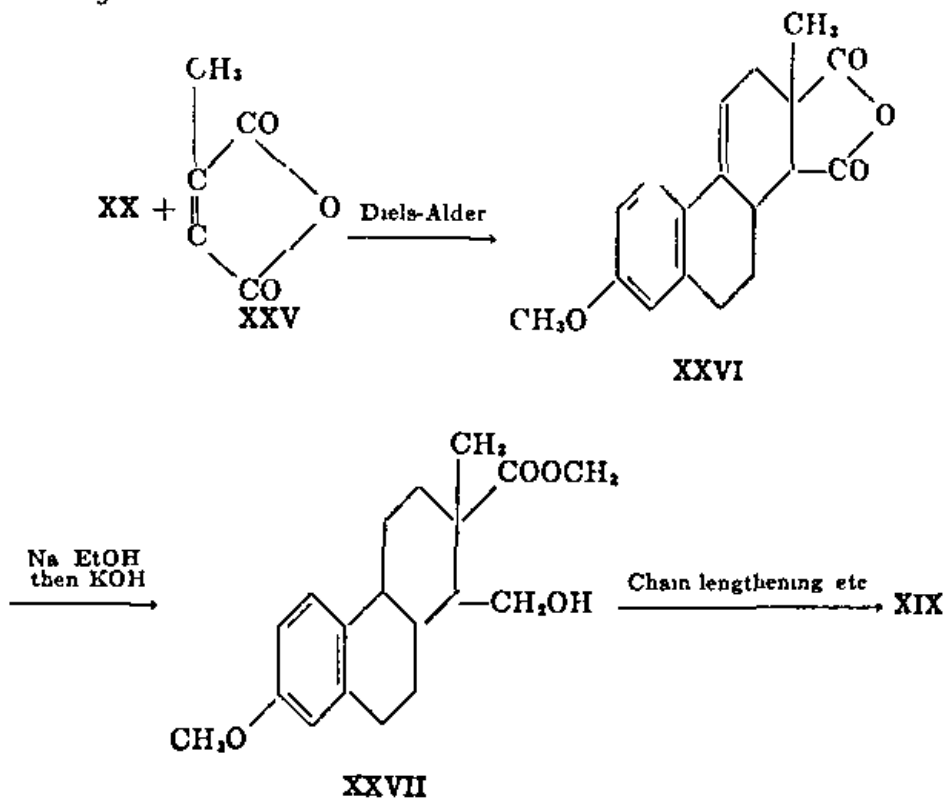


XXIII



XXIV

C Breitner Synthesis



The D ring is probably fused to the phenanthrene system in the *cis* configuration, in the native estrogens, rings C and D are believed to be in a *trans* relationship but this has not been rigorously proved On the other hand, it is possible that the condensation of XX and XXI did not proceed in the manner outlined, so that XXIV rather than XIX may actually be the product in hand

Breitner (128) sketchily described a method for the synthesis of estrone The initial step appears to be similar to that in the Dane and Schmidt synthesis XX was reacted with citraconic acid anhydride (XXV), the adduct, XXVI, on reduction with sodium and alcohol yielded a lactone, hydrolysis of the latter gave XXVII The procedure from this point on bears a resemblance to the Bachmann synthesis The final product possessed about the same degree of estrogenic potency as estrone but the physical properties are not identical Separation of stereoisomers was not carried out at any stage in the synthesis

3 *Some Interesting Homologs of the Estrogens*

Quite a number of interesting homologs of equilenin and estrone have been prepared and a few of these will be described here Antedating the Bachmann synthesis, Koebner and Robinson (98) synthesized *x*-nor-

equilenin[1] (the prefix *x* is used to indicate indeterminate stereochemical configuration and the prefix "nor" to indicate that the angular methyl group between rings C and D is absent), Weidlich and Meyer-Delius (186) consider the terminal rings of this compound to be *trans*-linked on the basis of comparative hydrogenation experiments in acid and alkaline solutions The acetate of *x*-norequilenin is estrogenic only in 10-mg doses (Koebner and Robinson, 98) An *x*-norestrone was prepared by Robinson and Rydon (148), its ring system probably has the *cis-cis* configuration Dane and Eder (38) synthesized an *x*-dehydronorestrone by a different route

Variations in the nature of the angular group of *dl*-equilenin have been introduced It appears that the estrogenic potency is largely preserved in the homologs up to *n* propyl, but the *n* butyl homolog is inactive (Bachmann and Holmes, 5) D-Homoequilenin (stereochemical configuration?) was prepared by Burnop *et al* (20), the D ring in this compound is six membered instead of five-membered as in the normal steroid series A partial synthesis of D-homoestrone has been described (Goldberg and Studer, 64), this compound has about one-thirtieth the estrogenic potency of estrone

Bachmann and Wilds (7) described the total synthesis of the stereoisomeric forms of *dl*-17-equilenone, i *e* equilenin derivatives lacking the 3 OH group The importance of this OH group for biological potency is emphasized by the fact that the *dl*-17-equilenones (α and β forms) are lacking in estrogenic activity (tested up to 500 μg in castrated female rats) Such compounds are also of interest metabolically in view of the recent isolation of 3-desoxyequilenin from pregnant mares' urine (Prelog and Führer, 141), this substance shows estrogenic activity at a 100–150 μg dose level The urinary steroid is dextrorotatory and has the same stereochemical configuration as that in native equilenin, the resolution of the *dl* 17-equilenones and comparison of these with the urinary product would complete the correlation of the two *dl* series Wilds *et al* (193) recently obtained evidence which indicates that the β form of 17-equilenone probably has a *trans* C D ring juncture, as is assumed to be the case in equilenin The α form of 17-equilenone possesses a *cis* C D ring juncture in all likelihood This opinion is shared by Birch, Jaeger, and Robinson (12), who synthesized by an independent route a product identical with α-17-equilenone (*dl* isoequilenin was also prepared) On the basis of results obtained with the model substance, α hydrindanone, this group of investigators is inclined to believe that equilenin (and probably the other hormones and sterols) has the *trans* configuration at the junction of rings C and D

[1] Synthesized by an independent route by Bachmann *et al* in 1943 (4)

B Partial Synthesis of Estrogens

1 *From Ergosterol*

In 1936, Marker *et al* (112) reported the partial synthesis of estrone (XIX) from dehydroneoergosterol (XXX) The latter is prepared by the method of Windaus by exposing a solution of ergosterol (XXVIII) and eosin to sunlight in the presence of oxygen A pinacone of ergosterol (Windaus and Borgeaud, 195) forms, which on heating loses the angular methyl group between rings A and B to give neoergosterol[2] (XXIX) In the latter compound, ring B is aromatic Further aromatization can be effected by dehydrogenation in the presence of platinum, dehydroneo ergosterol (XXX) (Honigmann, 82) is thereby obtained The latter compound is naptholic It is reduced with amyl alcohol and sodium to produce a phenolic steroid (XXXI) in which ring B is now saturated Removal of the side chain in this compound is effected by chromic acid oxidation resulting in the formation of estrone (XIX)

Windaus and Deppe (196) failed to duplicate the results of Marker and co-workers (112) They questioned the results of these investiga tors on the grounds that reduction of the naptholic ring of dehydroneo-ergosterol gives primarily nonphenolic material Marker (109) stated later that it was a minor product (phenolic) and not the major product (nonphenolic) which was subsequently utilized in the synthesis (see also Section I D 2) Unfortunately, experimental details for the partial synthesis of estrone were not furnished by Marker and his group To date, there has been no confirmation of this synthesis although two members of Marker's group independently duplicated his results

Remezof (145) obtained from neoergosterol (XXIX), by a procedure of oxidative degradation which was not very clearly described (lacking in characterization of the intermediary products), a nonphenolic isomer (XXXII) of estrone in which ring B instead of ring A is aromatic XXXII is claimed to be as potent as estrone

2 *From Cholesterol*

Inhoffen and co-workers (90) prepared dibromocholestanone (XXX-III) which on debromination gives a $\Delta^{1,4}$-dienone-3 The latter (XXXIV) on treatment with acetic anhydride and concentrated sulfuric acid yields a phenolic steroid (XXXV) The side chain is removed by chromic acid oxidation to give 1-methylestrone (XXXVI) Inhoffen *et al* (91–92) subsequently extended this study to steroids in the andro gen series (Androgenic substances can be prepared from cholesterol by oxidative processes) They prepared the dibromo derivative of the

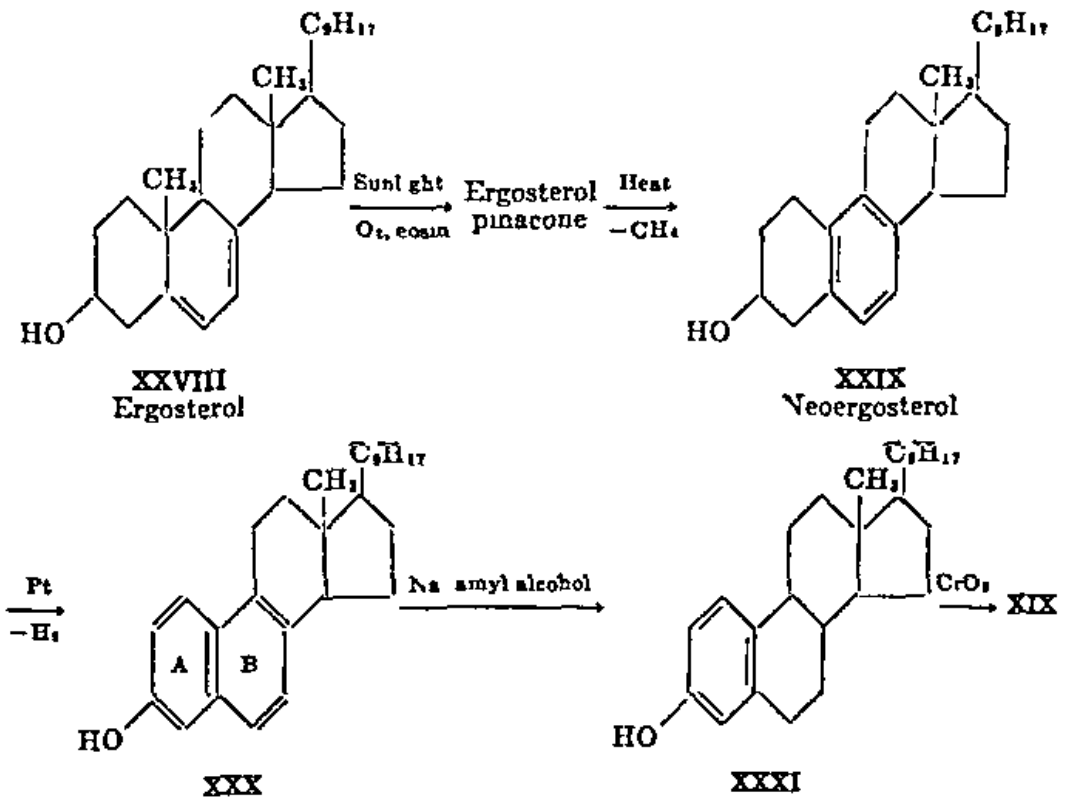
[2] For proof of structure see Inhoffen (89)

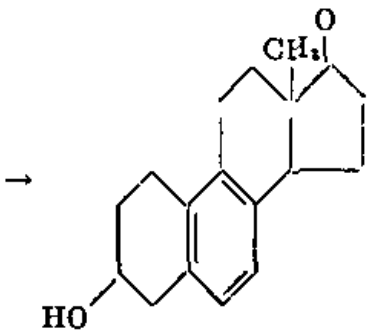
17-acetate of androstenol-17-one-3 This product on dehydrobromination yielded a $\Delta^{1,4}$-dienone-3 (XXXVII), which on treatment with acetic anhydride and sulfuric acid gave 1-methylestradiol (XXXVIII), it lacked estrogenic activity The dienone (XXXVII) was subjected to high temperature, methane was lost and a small amount of α-estradiol (XXXIX) was thereby obtained This synthesis correlates the aromatic steroid hormones with those of the nonaromatic series Apparently α-estradiol and testosterone (XL) have the same steric configuration at the points of fusion of rings B and C and also of rings C and D, the hydroxyl group at C-17 is *trans* to the methyl group at C-13 in both hormones But, as has been pointed out, (Wilds and Djerassi, 194) the possibility of inversion in the conversion of XXXVII to XXXIX is not ruled out since the reactions were carried out at a high temperature

Wilds and Djerassi (194) confirmed the work of Inhoffen utilizing essentially the same principles they improved the yield of α-estradiol considerably, and also more clearly defined the nature of the intermediary dibromo derivatives It has been reported (127) that as much as 15 kg estrone had been prepared from dehydroisoandrosterone (XLI) in 1944 by the Schering Corp in Germany The synthetic route resembles that

PARTIAL SYNTHESIS OF ESTRONE AND α-ESTRADIOL

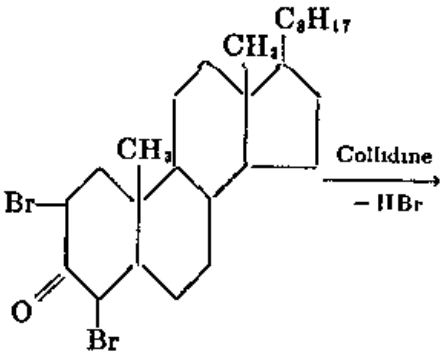
A Marker Synthesis



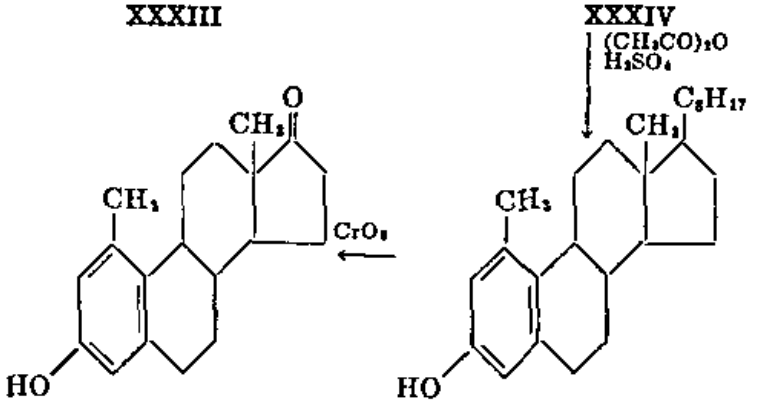


XXXII

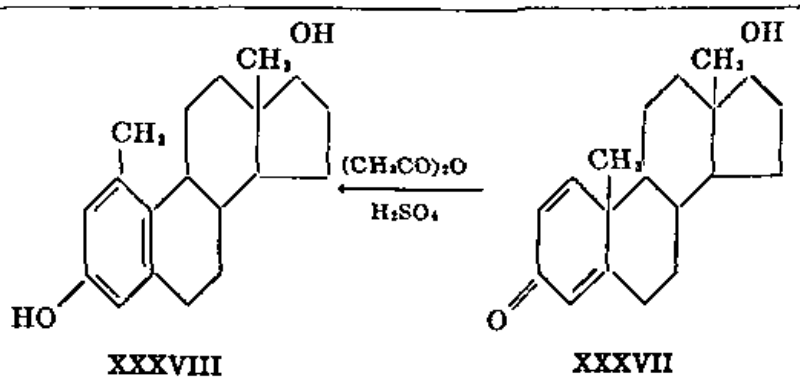
B Inhoffen (and others) Synthesis



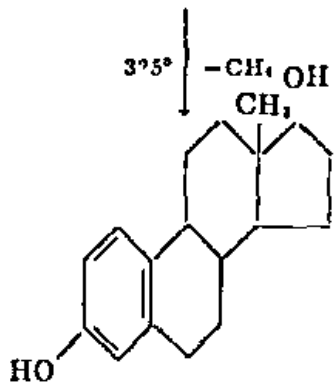
XXXIII XXXIV



XXXVI XXXV

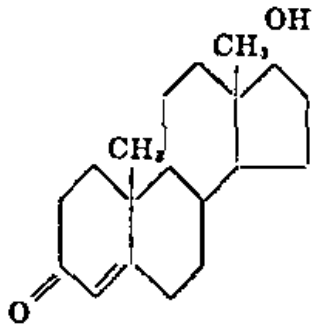


XXXVIII XXXVII

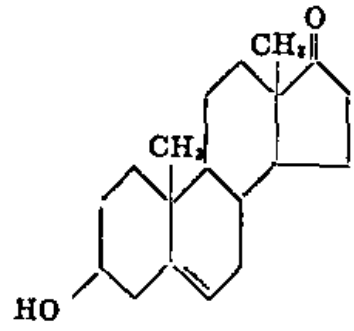


XXXIX
α Estradiol

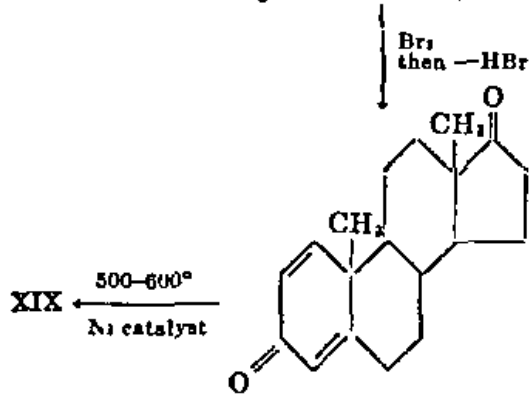
C Schering (Germany) Synthesis



XL
Testosterone



XLI
Dehydroisoandrosterone



XLII

of Inhoffen and is indicated below (XLI → XLII → XIX), experimental details have not been made available

C Estric Acids* and Related Products
Total and Partial Synthesis

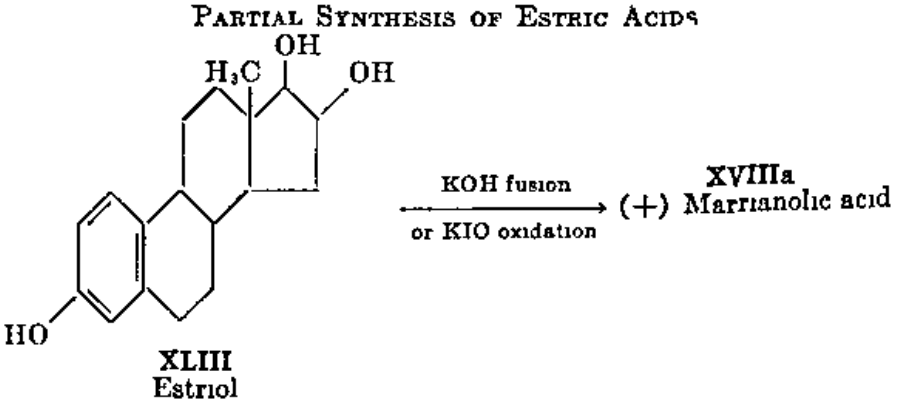
Estriol (XLIII) on fusion with potassium hydroxide yields a dicarboxylic acid, XVIIIa, which Miescher has named marrianolic acid

* Estrogenic carboxylic acids

This reaction was first studied by Marrian *et al* (121) and also by MacCorquodale *et al* (106,107) The methyl ether of the same dicarboxylic acid can also be obtained by the permanganate oxidation of the methyl ether of estriol in acetone (MacCorquodale *et al*, 106) Miescher (123) improved the yield by treating the benzyl ether of estriol with hypoiodite, the benzyl group being removed subsequently by hydrogenolysis Similar treatment (Heer and Miescher, 75) of the benzyl derivative of estrone (XIX) also yields marrianolic acid Heer *et al* (74) extended the study to equilenin (XIV) and obtained β(+)-bisdehydromarrianolic acid (XIa) Both types of marrianolic acids (XVIIIa and XIa) lack estrogenic activity Brief mention has been made by MacCorquodale *et al* (105) of other interesting acids and lactones which were obtained by them on more extensive degradation of the estrogens, none of these compounds were found by them to have any significant estrogenic activity, previous statements to the contrary notwithstanding (see also Thayer *et al*, 182)

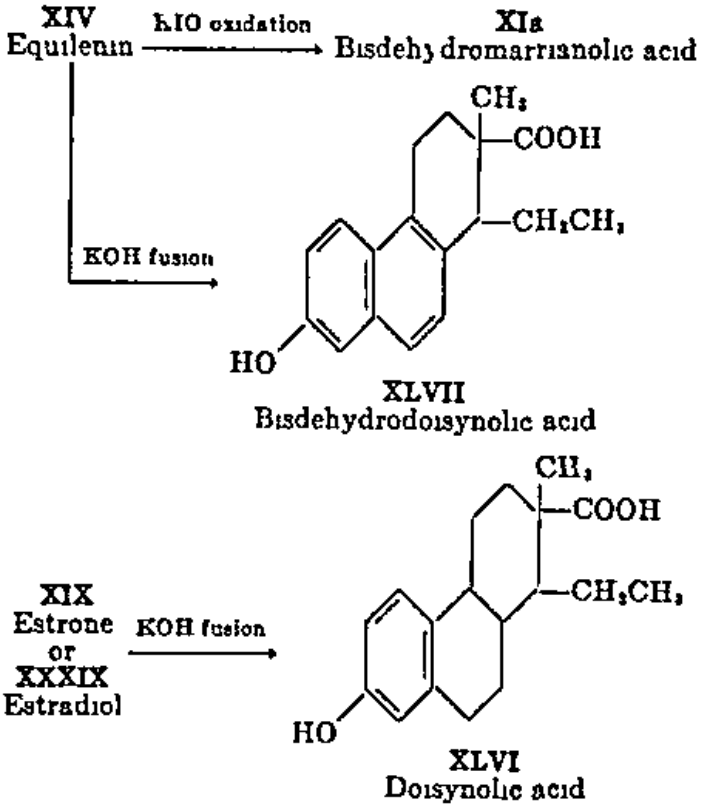
MacCorquodale *et al* (106) fused estrone (XIX) with potassium hydroxide and obtained a monocarboxylic acid (XLVI) Heer and Miescher (75) similarly fused estradiol (XXXIX) with potassium hydroxide and obtained a product identical with Doisy's Miescher named it doisynolic acid Doisynolic acid (XLVI) is a highly active estrogen when administered by the subcutaneous or oral route (W Hohlweg and H H Inhoffen, in 1937 and 1939 described patents for the preparation of monocarboxylic acids from estrogens and found these acids to be active orally) Heer *et al* (74) fused native equilenin (*d*-*n*-equilenin according to Bachmann *et al*) (and also dihydroequilenin) with potassium hydroxide and obtained a dextrorotatory and a levorotatory bisdehydrodoisynolic acid (XLVII) The levorotatory acid ("normal" or α) possesses an astonishingly high degree of estrogenic potency but the dextrorotatory ("iso" or β) acid is biologically inactive Miescher *et al* (74 123) synthesized bisdehydrodoisynolic acid, a task greatly facilitated by the work of Bachmann (see Section I, A, 1) VIII was

PARTIAL SYNTHESIS OF ESTRIC ACIDS



XLIII
Estriol

XVIIIa
Marrianolic acid



treated with magnesium ethyl bromide to give XLVIII, which was dehydrated, reduced, and demethylated to give bisdehydrodoisynolic acid (XLVII) as racemates of the *n*-(or α) and *iso* (or β) forms Rometsch and Miescher (149) succeeded in resolving the synthetic racemate of α-bisdehydrodoisynolic acid The $\alpha(-)$ bisdehydrodoisynolic acid was found to be identical with the levorotatory fusion product obtained from native equilenin, the $\beta(+)$ bisdehydrodoisynolic acid possessed about $\frac{1}{200}$ the estrogenic activity of the $\alpha(-)$ acid Anner and Miescher (2) more recently described a simplified synthesis of bisdehydrodoisynolic acid The bromide of III was condensed with the sodio derivative of propionyl propionic ester (XLIX) to give L, which was then cyclized to give XLVIII, the conversion of XLVIII to bisdehydrodoisynolic acid has been previously described Miescher and co-workers (1,11,75) have prepared a number of interesting homologs of bisdehydrodoisynolic acid, some of which are highly potent as estrogens $\alpha(-)$-Bisdehydrodoisynolic acid appears to be the most potent estrogenic substance thus far described, according to Miescher The estrogenic activity of this compound and related products are listed in Table I

Quite recently, Hunter and Hogg (88) described an elegant method for the total synthesis of doisynolic acid The starting product, *m* methoxyphenylacetic acid, was converted to the corresponding alcohol by reducing the ester with sodium and alcohol The alcohol was in turn converted to the bromide (XV) with the aid of phosphorus tribromide

The bromide was then condensed with ethyl-β-ketopimelate The condensation product underwent cyclodehydration with concentrated sulfuric acid Hydrolysis yielded a dibasic acid (XVI) which was converted to LI by the method of Bachmann *et al* (6) Treatment of LI with an equivalent amount of ethyl magnesium iodide yielded the ethylidine derivative, LII, catalytic reduction, followed by hydrolysis and demethylation gave a diastereoisomeric mixture of doisynolic acid (XLVI) which possessed a very high degree of estrogenic potency

TABLE I*

COMPARATIVE BIOLOGICAL POTENCY OF THE ESTRIC ACIDS AND ESTROGENIC HORMONES

Compound tested	Effective dose levels for vaginal response (rats A D test)	
	Subcut (μg)	Oral (μg)
Native estrogens		
Estrone (XIX)	0 7	20 to 30
α-Estradiol (XXXIX)	0 3 to 0 4	20 to 30
Equilenin (XIV)	10 to 20	
Stilbestrol[b] (LIV)	0 3 to 0 4	0 7 to 1 0
Doisynolic acids (and homologs)		
Doisynolic acid (from α-estradiol) (XLVI)	0 7 to 1 0	1 5
Synthetic rac doisynolic acid (XLVI)	0 8 to 0 9[c]	
Synthetic rac α bisdehydrodoisynolic acid (XLVII)	0 1 to 0 15	0 1 to 0 2
α(−) Bisdehydrodoisynolic acid (from equilenin) (XLVII)		0 05 to 0 1
LV (racemates)		
$R_1 = CH_3$ $R_2 = OH$	0 1 to 0 2	0 1 to 0 2
H OH	> 100	> 100
C_2H_5 H	5	5 to 10
Marrianolic acids		
Marrianolic acid (XVIIIa)	> 100	
α Bisdehydromarrianolic acid (XIa)	> 1000	

* Data (except when indicated otherwise) compiled by Miescher *et al* (2 74 75 123)

[b] For a recent comprehensive review on synthetic nonsteroid estrogens see Solmssen (173)

[c] Data by Hunter and Hogg (88)

Anner and Miescher (2) confirmed the work of Hunter and Hogg in that they also succeeded in preparing the same ethylidine compound (LII) The former group of investigators observed that this compound readily undergoes rearrangement to bisdehydrodoisynolic acid, this fact

led Anner and Miescher to suspect that the highly estrogenic but uncharacterized product which Hunter and Hogg obtained on hydrolysis and demethylation of LII is probably bisdehydrodoisynolic acid (XLVII) Anner and Miescher succeeded in partially hydrogenating the ethylidine compound (LII) to give monodehydrodoisynolic acid (LIII) As might be expected, due to the fact that the double bond is situated between two tertiary carbon atoms, monodehydrodoisynolic acid is relatively resistant to hydrogenation It was, nevertheless, converted to a racemic mixture of doisynolic acids, as a by-product, bisdehydrodoisynolic acid was obtained in small yield This observation is reminiscent of the reaction

TABLE II

NATIVE ESTROGENS RELATIVE AND ABSOLUTE POTENCY*

Absolute activity (vaginal response in spayed adult rodents)

Compound	μg per		
	Rat unit		Mouse unit
Estrone (XIX)	1 0	1 0	0 125
α-estradiol (XXXIX)	0 08	0 125	0 05
β-estradiol (LVI)	3 3	12 5	1 25
Reference	190	132	25

Relative activity

Compound	Spayed rat method	Immature mouse-uterine weight method
Estrone (XIX)	100	100
α-estradiol (XXXIX)	1000	300
β estradiol (LVI)	10 (very irregular)	7 5
Estriol (XLIII)	20 (very irregular)	40
Equilin (LVII)	*ca* 25	11 0
Reference	97	97

* See also Table I

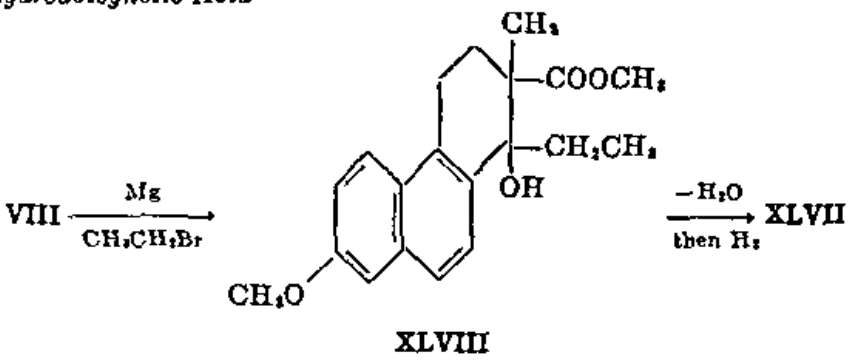
whereby isoequilin A (LXXIII) is converted to 14-epiequilenin (Hirschmann and Wintersteiner, 81), analogous also is the disproportionation reaction whereby dihydroequilin is converted into 8-isoestradiol and dihydroequilenin (see Section I, D, 3) It is not well established in the opinion of Anner and Miescher whether the estrogenic potency of the doisynolic acids thus obtained might be due to contamination with bisdehydrodoisynolic acid since some of the latter is formed concomitantly

Heer and Miescher (75) have attempted the arduous and bewildering

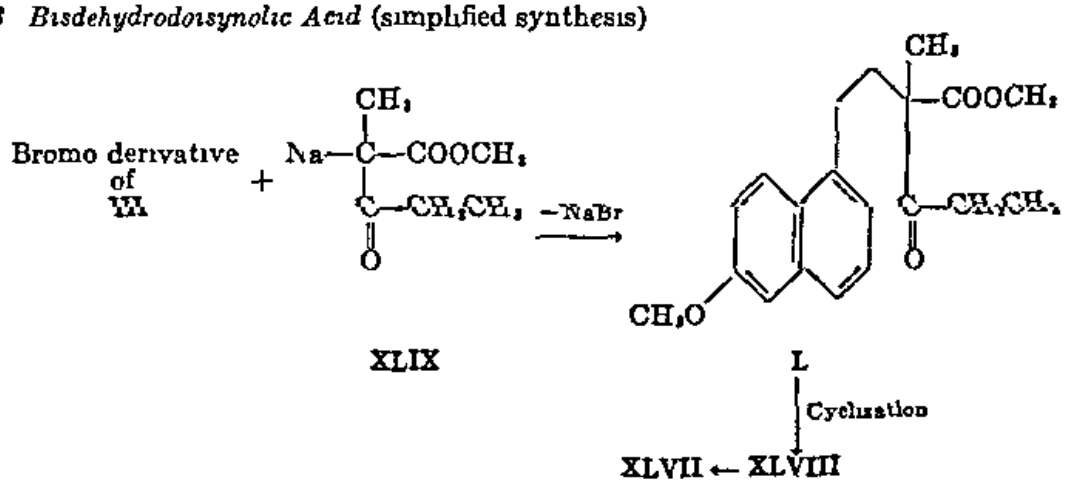
task of correlating the spatial configuration in the estric acids with one another and with that in the native or natural hormones The pertinent facts are presented forthwith In the course of total synthesis of equilenin, Bachmann *et al* (3) obtained two racemic mixtures of bisdehydro marrianolic acid * The conversion of these compounds to equilenin (and iso-equilenin) and resolution into its antipodes are indicated in Chart 1 It is curious that an inversion in optical rotation occurs in the process of converting the α bisdehydromarrianolic acids into the iso forms of equilenin The (+) bisdehydromarrianolic acid (β or "normal"), which may be obtained from native equilenin, is probably identical with the product derived from total synthesis by Bachmann *et al* (3), this is based on a comparison of the melting points of the derivatives of the first product with the corresponding derivatives of the $\alpha(+)$ $\alpha(-)$ and β (racemate) of the bisdehydromarrianolic acids prepared by total synthesis Heer and Miescher (75) have correlated some of the compounds of the marrianolic acids series with the corresponding compounds of the doisynolic acid series This was accomplished by selective replacement

TOTAL SYNTHESIS OF ESTRIC ACIDS*

A *Bisdehydrodoisynolic Acid*

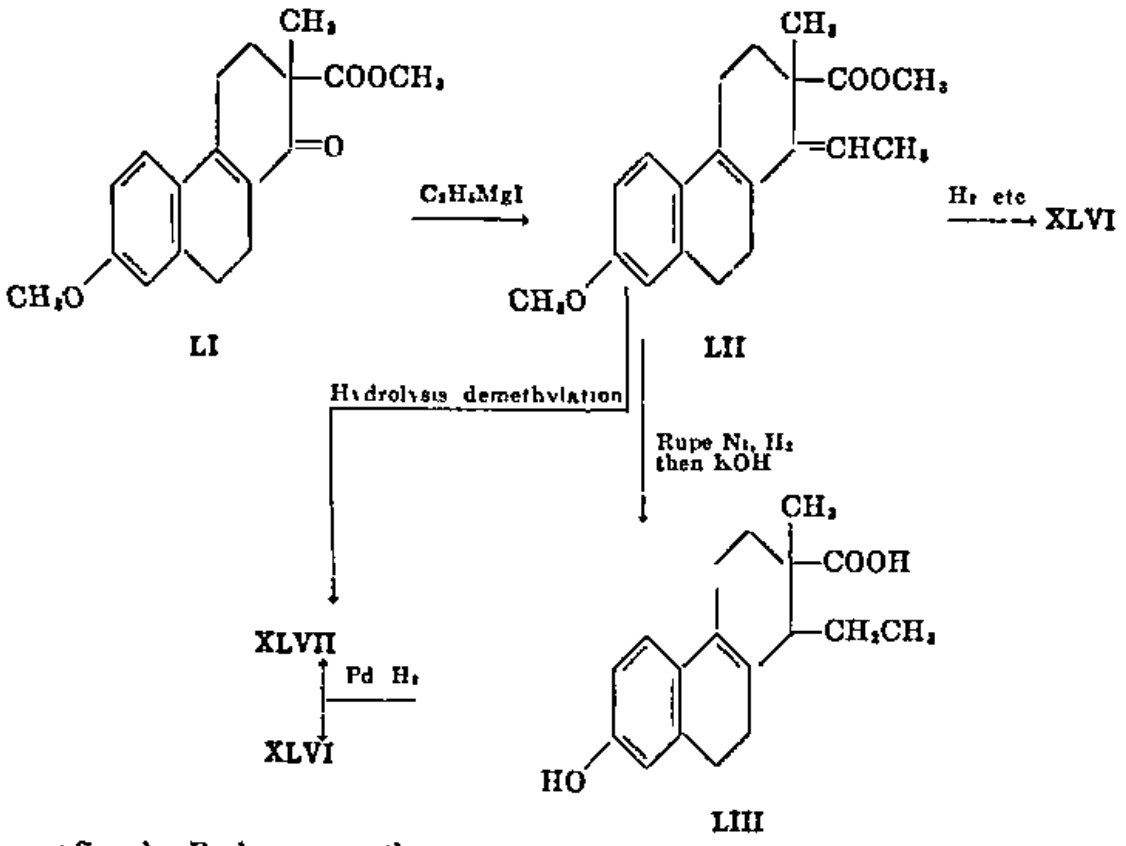


B *Bisdehydrodoisynolic Acid* (simplified synthesis)



* As the methyl ethers

C Doisynolic and Monodehydrodoisynolic Acids



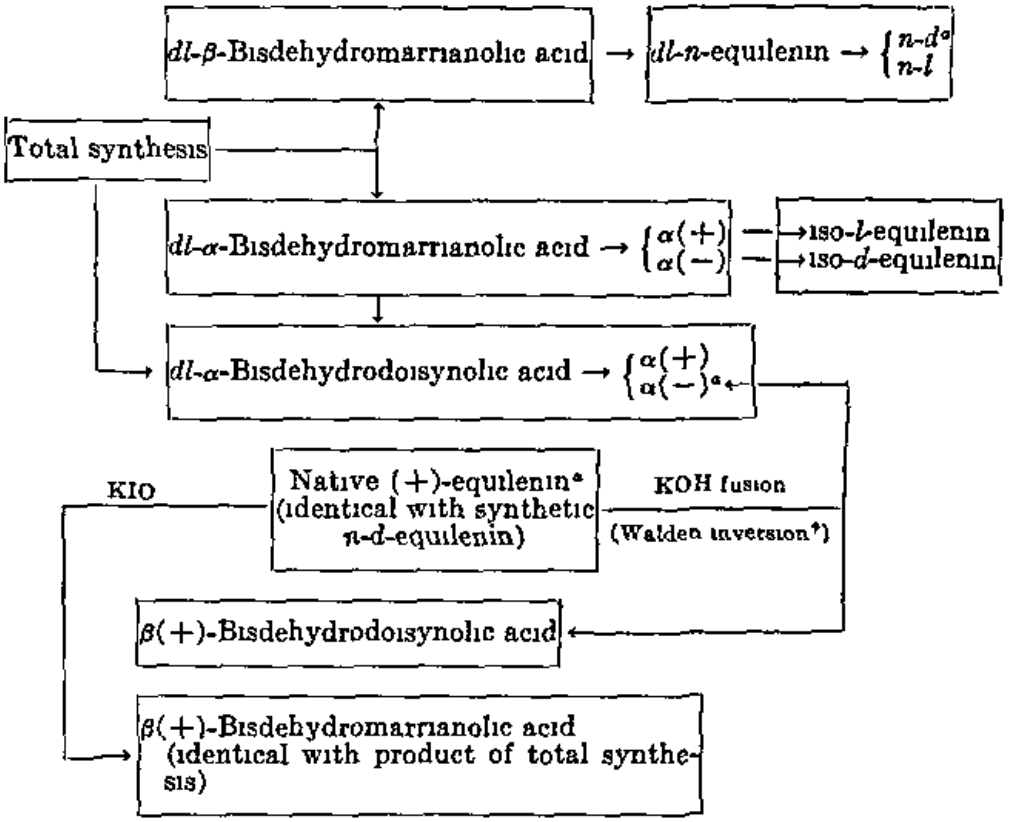
• See also Bachmann syntheses

of the hydroxyl group with chlorine in the acetic acid group in XVIII, Rosenmund reduction to the aldehyde, and Wolff Kishner reduction (XLVI) These authors also described the preparation of the estric acids of the lumi series, using lumiestrone (see Section I, D, 6) as the starting compound It is curious that an inversion in optical rotation occurs when lumiestrone is converted into lumimarrianolic acid The chemical interconversions of the above-mentioned compounds and of other related hormone products (see Section I, D) are diagrammatically indicated in Charts 1 and 2 It is rather difficult to explain why the α-bisdehydromarrianolic acids (biologically inactive) yield a biologically active α-bisdehydrodoisynolic acid, since the isoequilenins which the former compounds also yield are biologically inactive The picture is complicated by the finding that potassium hydroxide fusion of native equilenin results in the formation of two bisdehydrodoisynolic aids (one belonging to the α series, the other to the β series), whereas similar treatment of estrone yields only one product, the drastic conditions of alkaline fusion may have induced a Walden conversion in the former instance Heer and Miescher finally submit for consideration the following conclusions based on the findings summarized in Charts 1 and 2

(*a*) If native estrone and equilenin possess a *trans* C:D ring juncture, then, in α(−)-bisdehydrodoisynolic acid ("normal" or biologically active), the 1-ethyl group and the 2-carboxyl group are in cis relationship;

CHART 1

ESTROGENIC HORMONES AND ESTRIC ACIDS CHEMICAL INTERCONVERSIONS



* Biologically active

conversely, in β(+)-bisdehydrodoisynolic acid ("iso" or biologically inactive), these groups are in *trans* relationship:



(Phenanthrene system of numbering)

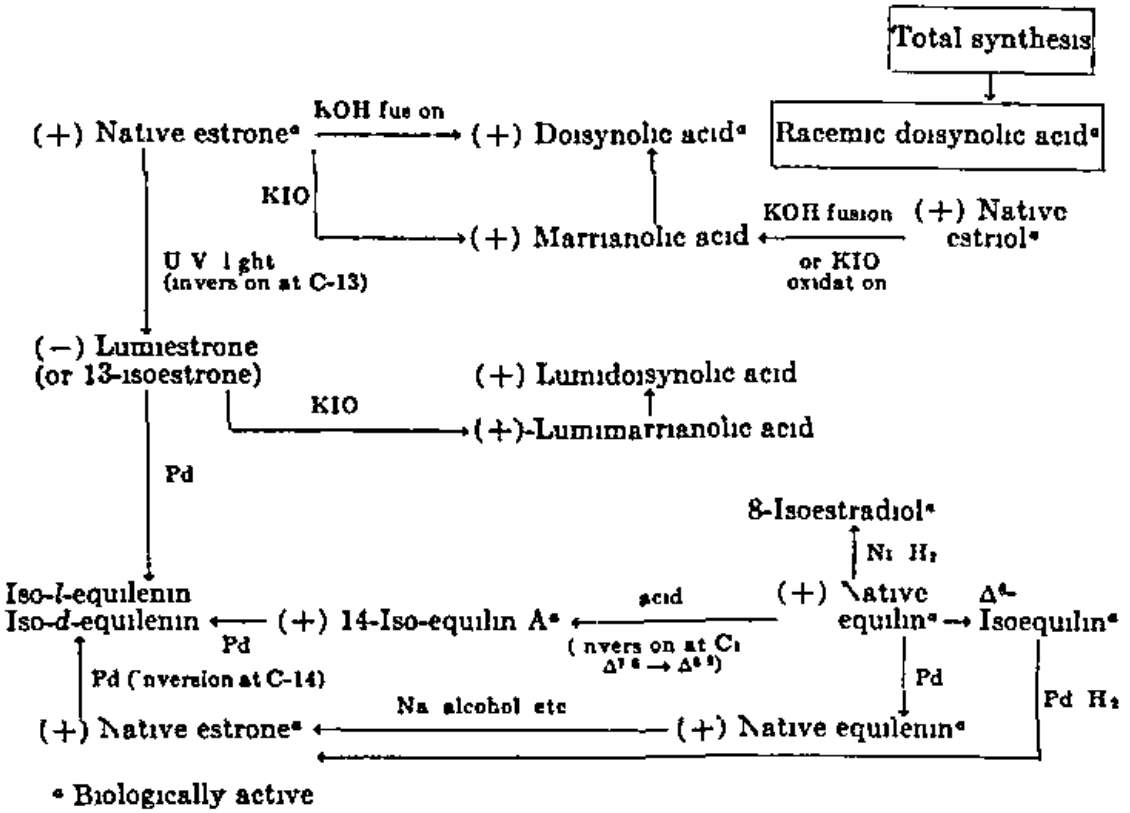
(*b*) If the stereochemical relationships are the reverse of those stated above, it follows that the C·D ring configuration is not identical in estrone and equilenin *

* But the C D ring configuration in estrone and in equilenin appears to be identical since (*a*) Marker (109) converted dihydroequilenin to α-estradiol (an observation which has, however, not been confirmed), (*b*) equilin is readily dehydrogenated to

Heer and Miescher allow for a possible inversion of the 1-ethyl group in the course of the conversion of the marrianolic acid series to the doisynolic acid series, but they consider this to be rather unlikely

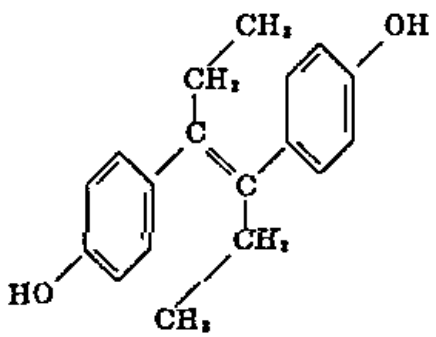
CHART 2

ESTROGENIC HORMONES AND ESTRIC ACIDS CHEMICAL INTERCONVERSIONS

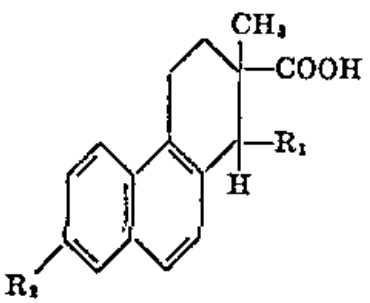


* Biologically active

In any event, these authors feel that final proof for the stereochemistry of the estric acids (and of the native hormones) will require further experimentation



LIV
Diethylstilbestrol



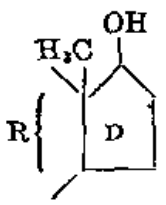
LV

equilenin (47) equilin was converted by Pearlman and Wintersteiner (135 136) to estrone

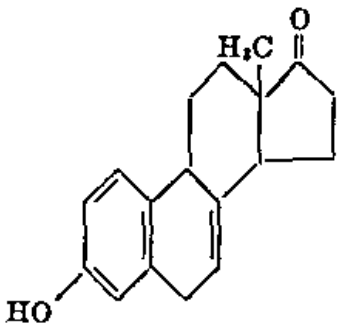
D Chemical Reactivity of Estrogens

1 *Reactions at C-17 Only*

Schwenk and Hildebrandt (159) catalytically reduced estrone (XIX) and obtained two epimeric diols, full experimental details were not given Wintersteiner *et al* (197) more fully characterized the diols, the 17-α-hydroxy compound (XXXIX) is readily precipitated with digitonin, a reaction which appears to be unique in the estrogen series Butenandt and Goergens (25) independently reported the preparation of α and β-estradiol (XXXIX and LVI) from estrone by catalytic reduction of the latter in the presence of a nickel catalyst Other methods for the reduction of the carbonyl group in estrone have been described, in every instance, however, α-estradiol is the predominant reduction product Hydrogenation of a neutral alcoholic solution of estrone in the presence of platinum oxide will result in a 90% yield of α-estradiol (Marker and Rohrmann, 115c) Methods for the reduction of estrone with the aid of sodium and alcohol have been reviewed by Whitman *et al* (192) who reduced estrone in 10% potassium hydroxide with the aid of Raney nickel The reduction of estrone by the Meerwein Pondorff method, in which aluminum isopropoxide is employed, gives a mixture of α and β estradiol in which the content of the β epimer is appreciable (Marker and Rohrmann, 115a)



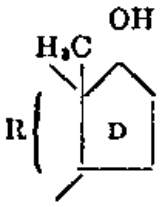
LVI
β-Estradiol (see XXXIX)



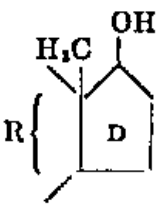
LVII
Equilin

The diols obtained from equilin (LVII) and equilenin have been described David (40) reduced equilin in alcohol with sodium, and obtained a diol α dihydroequilin The reduction of equilin by the Meerwein-Pondorff method gives predominantly α-dihydroequilin, the β-isomer failed to be isolated (unpublished observations, Pearlman and Wintersteiner) Equilenin on reduction with sodium and alcohol, yields α-dihydroequilenin (David 40) The Meerwein-Pondorff reduction of equilenin was first studied by Marker *et al* (113), who succeeded in

obtaining α- and β-dihydroequilenin (LVIII and LIX) Equilenin may also be hydrogenated in neutral alcohol in the presence of platinum oxide to give α dihydroequilenin (Marker and Rohrmann, 115b), the latter is also obtained on catalytic hydrogenation of XIV in an acid medium if the hydrogenation is not permitted to proceed further (Ruzicka *et al*, 151)



LVIII
α-Dihydroequilenin



LIX
β Dihydroequilenin

(see XIV Equilenin)

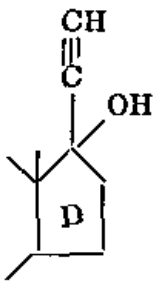
The 17-α-diols in the estrogen series are considerably more active than the 17 carbonyl compounds from which they are derived On the other hand, β estradiol is appreciably less active than estrone Tables I and II list the native estrogens and their estrogenic potency A strict comparison of the estrogenic activity given in the literature cannot be made because assay procedures vary from laboratory to laboratory

The 17 hydroxyl group in the α diols of the estrogen series is believed to be in *trans* configuration with respect to the angular methyl group on carbon 13 (Wintersteiner in 37) A correlation of this configuration in the estrogen series has been established with that of the 17 α-hydroxy compounds in the androgen series (see Section I B 2) The 17-β diol in both the androgen and estrogen series can be dehydrated, by way of the 17-benzoate to yield the corresponding Δ^{16}-derivative, the 17-α-hydroxyl compounds are resistant to dehydration, however When estriol is heated with potassium hydrogen sulfate in a high vacuum, a molecule of water is lost and estrone is formed (Butenandt and Hildebrandt, 26, Marrian and Haslewood 122)

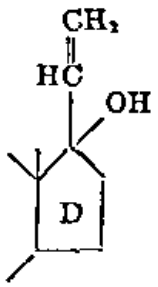
The 17-hydroxylated estrogens may be oxidized to the corresponding 17-ketones with the aid of chromic anhydride (for example, see 25), the phenolic hydroxyl group must, of course be protected by acylation or methylation Oppenauer oxidation of the diols is probably the most elegant method to achieve this aim, inasmuch as protection of the phenolic hydroxyl group is not necessary and there results little or no destruction of estrogenic material (132)

Estrone reacts with acetylene to form an addition compound designated as 17 ethinylestradiol It is highly estrogenic (0 1 μg ≅ 1 rat unit on subcutaneous injection, 3 μg ≅ 1 rat unit on oral adminis

tration) Ethinylestradiol may be partially hydrogenated in the



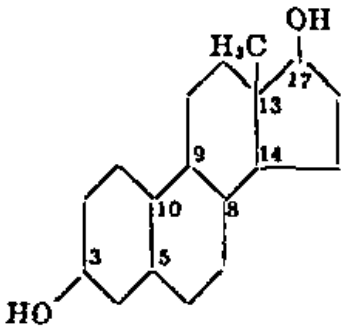
presence of Rupe nickel to give the 17-ethenyl derivative The latter compound is less potent that 17-ethinylestradiol by the subcutaneous route and considerably less so by the oral route (Inhoffen *et al*, 90a)



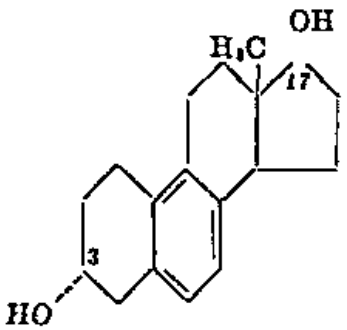
2 *Reduction of Aromatic Nucleus*

a Estrone or Estradiol In 1930 Butenandt (22) observed that catalytic reduction of estrone in a neutral medium resulted in the reduction of the aromatic ring, a monohydroxyestrane derivative was isolated which was considered to be an estranol-3, but Marker and Rohrmann (115c) suggest that this is probably an estranol-17 Marker and Rohrmann (115c) also believe that the reaction was an aberrant one probably due to traces of alkali in the Adams catalyst employed Schoeller *et al* (157) catalytically hydrogenated estrone (or estradiol) and obtained a complex mixture of isomeric octahydroestrones (LXX) lacking in estrogenic activity In 1936 Dirscherl (46) made a detailed study of the results obtained on catalytic hydrogenation of estrone in an acid medium He isolated two isomeric estranediols (LXX), one of these is identical with the estranediol B which Marker *et al* (116,117) obtained from the urine of nonpregnant women Reduction of estrone generates new asymmetric centers at C-3, C-5, C-10 and C-17 Marker's estranediol A and B (both isolated from urine) differ in the configuration of the hydroxyl group at C-3 or C-17 since both compounds yield the same diketone on oxidation (116,117) Dirscherl (46) also isolated as by products from the hydrogenation of estrone, two monohydroxyestrane derivatives lacking an hydroxyl group at C-3

b Equilenin or Dihydroequilenin $\Delta^{6,7,9}$-estratrienediol-3,17(α) (LXXI) is obtained from equilenin on reduction with sodium and alcohol (David, 41, Marker *et al*, 118, Ruzicka *et al*, 151), the diol may be obtained in approximately 80% yield (151) A diol epimeric at C-3 has been obtained as a by-product on hydrogenating equilenin in acidic alcohol in the presence of platinum oxide, a *cis* configuration for the C-3-



LXX
Estranediol
(asymmetric centers are numbered)



LXXI
(LXXII lacking OH at C_3)

OH group is favored (151) The major product in the latter instance is $\Delta^{6,7,9}$-estratrienol-17(α), this monohydroxy compound (LXXII) was first prepared by Marker *et al* (113,114) from equilenin in 70% yield by essentially the same procedure This substance can be similarly prepared from α-dihydroequilenin (Marker and Rohrmann 115)

On treatment of dihydroequilenin in boiling *n* amyl alcohol with sodium, there are formed nonphenolic products in about 75% yield and phenolic products in about 20% yield (Marker, 109), α-estradiol can be obtained from the phenolic fraction if α-dihydroequilin is the starting product, and similarly β-estradiol if β dihydroequilenin is substituted in the reaction The nonphenolic products have been described above

3 *Some Chemical Studies on Equilin*

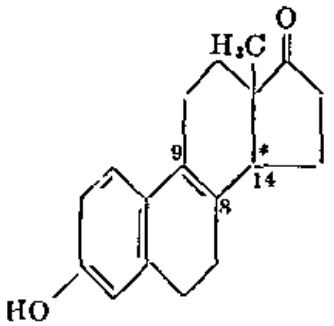
Equilin is resistant to catalytic hydrogenation with palladium, instead, dehydrogenation readily occurs with the result that equilenin is obtained (Dirscherl and Hanusch, 47) Serini and Logemann (165) confirmed this observation, they also observed that on treatment of dihydroequilin with hydrogen in the presence of Raney nickel, a disproportionation reaction occurs which involves no uptake of hydrogen The products formed are dihydroequilenin and isoestradiol The latter is believed to be isomeric with α-estradiol with respect to the configuration at C-8, the stereoisomer is not precipitable with digitonin Chromic acid oxi

dation of 8 isoestradiol gives 8 isoestrone The iso compounds have about one third the biological activity of the corresponding estrogens

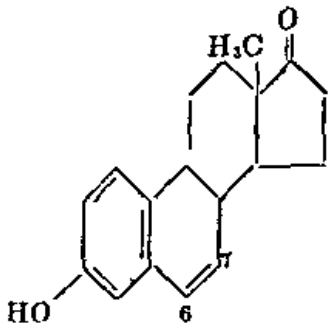
Hirschmann and Wintersteiner (81) treated equilin with hydrochloric acid and acetic acid and obtained 14-epi-$\Delta^{8,9}$-equilin (designated 14 iso-equilin A, LXXIII), inversion at C-14 occurred in the process of shifting the double bond from the 7,8 position The latter substance when heated with palladium yielded 14-epiequilenin (for the total synthesis of this compound, see Section I, A, 1)

Another isomer of equilin has been prepared by Pearlman and Wintersteiner (136), the double bond is located between positions 6 and 7 The Δ^6 isomer (LXXIV) is obtained from 7-hydroxyestrone (LXXV) by eliminating hydrochloric acid from the intermediary 7-chloro derivative Δ^6 Iso-equilin possesses about one third the physiological potency of estrone in contrast to equilin, the double bond isomer is readily con verted to estrone on catalytic hydrogenation

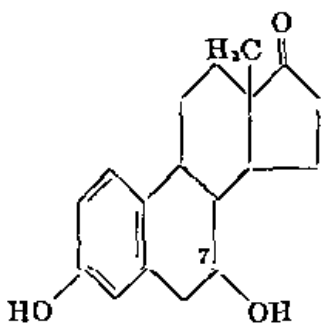
The hydroxylation of the double bond in equilin is described below (Section I, D, 4 a)



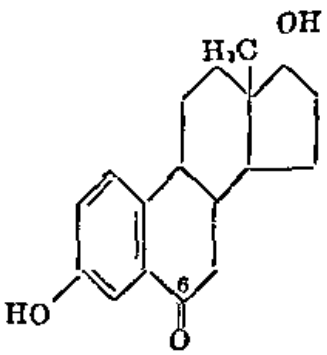
LXXIII
14-Isoequilin A (* inversion)



LXXIV
Δ^6-Isoequilin



LXXV
7 Hydroxyestrone



LXXVI
6-Keto-α-estradiol

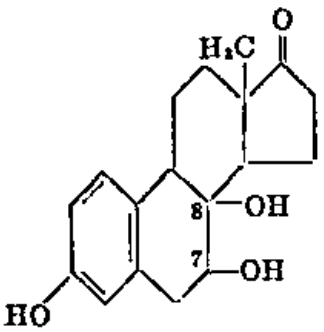
4 *Oxygenated Derivatives of Estrogens*

a Oxygen in Ring B Estradiol, on oxidation with chromic acid, yields 6-keto-α-estradiol (LXXVI) (Longwell and Wintersteiner, 104), the hydroxy groups are protected by preparing the diacetyl derivative

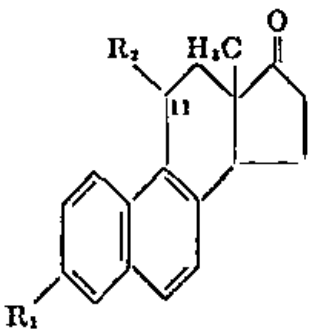
The 6-keto derivative has about one fourth the estrogenic activity of α-estradiol 6-Ketoestrone may be similarly prepared from estrone (Schwenk, 158)

Equilin on treatment with osmium tetroxide, yields a 7 8 glycol (LXXVII) which is inactive as an estrogen even at a 500 μg level (Serini and Logemann 165) When the glycol is distilled in a high vacuum, dehydration occurs and 7 ketoestrone is obtained (Pearlman and Wintersteiner, 135) Catalytic reduction of the 7 keto compound yields 7 hydroxyestrone Both 7 keto and 7 hydroxyestrone possess about the same estrogenic potency which is about $\frac{1}{300}$ that of estrone

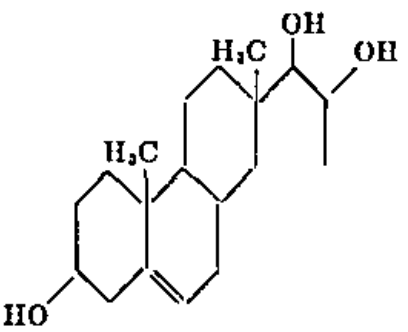
b Oxygen in Ring C Chromic acid oxidation of equilenin acetate will yield the corresponding 11 keto derivative (LXXIX) (Marker and Rohrmann 115b)



LXXVII
Equilin glycol



LXXIX R = OH R_1 = O
LXXIX a R_1 = H R_2 = H
LXXIX b R = H R_2 = O



LXXX
Urinary androstenetriol

c Oxygen in Ring D Partial Synthesis of Estriol and Its Stereoisomers Quite recently the conversion of estrone to estriol and some of its stereoisomers has been realized Huffman *et al* (83 84) prepared the benzoyl derivative of 16-oximinoestrone the methyl ether derivative was previously described by Litvan and Robinson (102) The former group of workers effected a reductive hydrolysis of the oximino group with zinc and acetic acid, the corresponding ketol was thereby obtained and on catalytic reduction it yielded isoestriol A Subsequently, Huffman and Miller (86) announced the preparation of a triol identical with naturally occurring estriol, but no experimental details were furnished Huffman (83) also prepared the methyl ether of 16-ketoestrone by a gentle oxidation of the methyl ether of the ketols derived from estrone

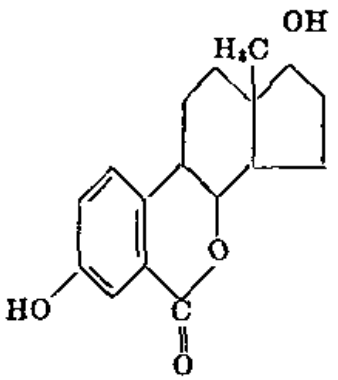
Attempts have been made to establish the spatial relationship of the 16 17 OH groups in estriol with each other and with those in the androstenetriol (LXXX) first isolated by Hirschman (79) from urinary sources Using the same procedure for converting estrone into estriol Huffman and Miller (86) were successful in preparing Hirschmann s triol from

dehydroisoandrosterone (XLI) The spatial arrangement of the hydroxyl groups in androstenetriol and in estriol may therefore be assumed to be identical Huffman and Lott (85) cite indirect evidence which points to a *trans* geometric relationship between the vicinal hydroxyl groups in both triols This conclusion is in agreement with that reached by Ruzicka *et al* (142,152), who suggested that the vicinal groups in estriol are 16(β) and (17α) on the basis of their experiments in preparing stereoisomers of estriol These authors (142) dehydrated β-estradiol by way of its benzoyl derivative, a double bond was thus introduced between positions 16 and 17 This substance (Δ^{16}-estrone) on treatment with osmium tetroxide yielded a glycol which, considering its manner of derivation, is probably 16(α),17(α) This triol is not identical with estriol nor with isoestriol A Estriol, isoestriol-16(α),17(α) and Δ^{16} estrone are active as estrogens at 10-μg , 5–10-μg , and 40–50-μg dose levels, respectively Ruzicka *et al* (152) similarly prepared an androstanetriol starting with Δ^{16} androstanol-3(β) This triol is stereoisomeric with the hydrogenation product obtained from Hirschmann's triol

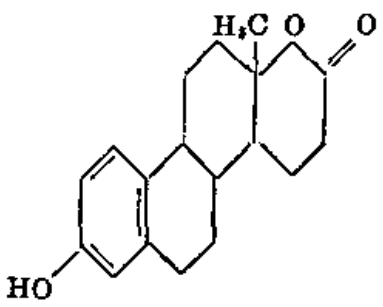
5 *Ring Splitting*

a Ring B Longwell and Wintersteiner (104) obtained a keto lactone (LXXI) as a by product in the oxidation of α-estradiol diacetate with chromic acid The estrogenic activity was not reported

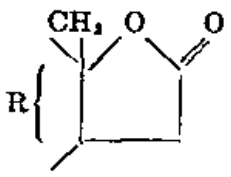
b Ring D A number of mono- and dicarboxylic acids may be obtained by fusing the native estrogens with potassium hydroxide or by



LXXXI



LXXXII
Westerfeld s lactone



LXXXIII
(see LXXXII)

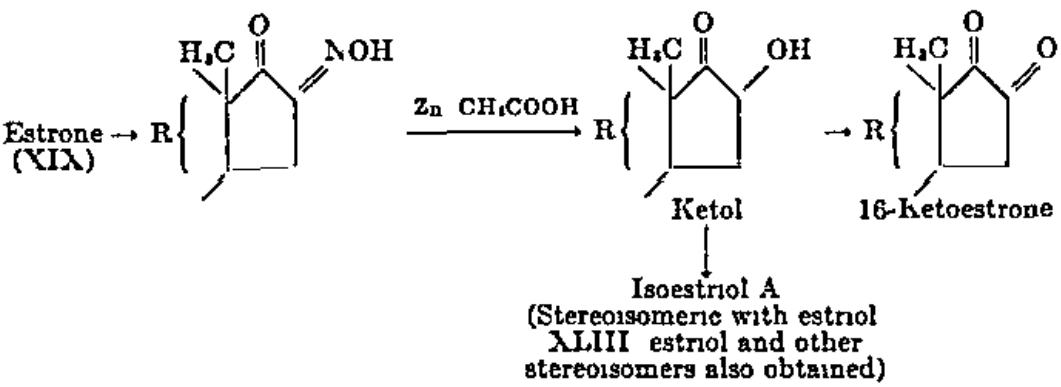
treatment with hypoiodite, permanganate, etc (see Section I, C) Westerfeld (188) obtained a lactone (LXXXII) on treating estrone with hydrogen peroxide in aqueous alkaline solution It possesses about $\frac{1}{14}$ the estrogenic activity (*i e* in its effect on vaginal cornification) of estrone but is more potent than estrone in its stimulating action on the pituitary (Smith, 170,171) In Doisy s laboratory (106) a closely related lactone was obtained on permanganate oxidation of the methyl ether of estriol, a tentative structural formula (LXXXIII) is given

6 *Irradiation*[3]

On irradiation of estrone with ultraviolet light inversion occurs at C-13, the product thus obtained has been named lumiestrone (Butenandt *et al* , 31) It is inactive as an estrogen even at a 100 μg level [4] It is interesting that the carbonyl group in lumiestrone shows marked steric hindrance, as, for example, in its behavior toward ketone reagents Dehydrogenation of lumiestrone with palladium black results in the formation of iso-l-equilenin, identical with the product synthesized by Bachmann *et al* (3) When estrone is similarly dehydrogenated iso-*d* equilenin is obtained, it is identical with the 14-epiequilenin of Hirschmann and Wintersteiner (81) Apparently inversion at C-14 occurs on dehydrogenation of estrone Butenandt *et al* (24) subsequently obtained additional support for the steric configuration of lumiestrone Estrone was irradiated with monochromatic light of 313-mμ wavelength The energy relationship was carefully studied and it was concluded that the photochemical conversion of estrone to lumiestrone is a unit quantum process, the 17 keto group is essential for the transformation since it alone absorbs light at 313 mμ (see lumiandrosterone, 29)

Partial Synthesis of Estriol and Stereoisomers[3]

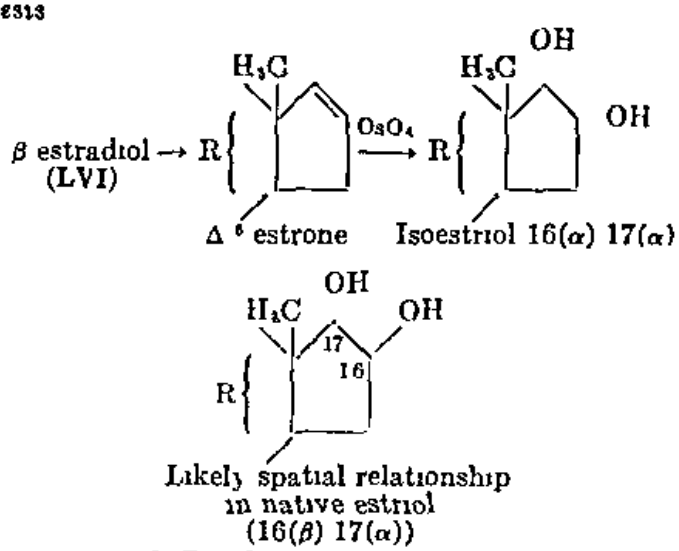
A Huffman Synthesis



[3] See Chart 2 page 371

[4] Figge (57) has obtained evidence which indicates that irradiated estrone may have a stimulating action on the pituitary crystalline lumiestrone however was not tested

B Ruzicka Synthesis



[a] D ring alone represented R indicates rest of structure as in estrone

II Metabolism of Estrogens

A Where Are Estrogens Formed?

Modification in sexual function or in the secondary sex characteristics of the organism may be correlated with the extirpation of certain endocrine organs or with changes in the morphology of these tissues Similarly, the rate of excretion of estrogens under normal and pathological conditions may give evidence of an indirect nature as to the ultimate source of estrogen elaboration For example, estrogen excretion rises markedly during the course of pregnancy and drops precipitously with the termination of pregnancy but the removal of the ovaries of pregnant women and of mares does not result in disappearance of estrogens from the urine The placenta is therefore implicated as a source of estrogen Studies of this sort have been reviewed previously (49,139) and will not

TABLE III

Isolation of Crystalline Estrogens from Likely Sites of Synthesis

Estrogen	Organ	Investigator
α Estradiol	Ovaries (sow)	MacCorquodale *et al* (108)
Estrone[a]	Ovaries (sow)	Westerfeld *et al* (191)
Estrone	Placenta (human)	Westerfeld *et al* (190)
α-Estradiol	Placenta (human)	Huffman *et al* (87)
Estriol	Placenta (human)	Browne (19)
Estrone	Adrenals (beef)	Beall (8)
Estrone	Testes (stallion)	Beall (9)
α-Estradiol	Testes (stallion)	Beall (9)

[a] Demonstrated but not isolated

be discussed further Such indirect evidence complements the more direct evidence furnished by the actual isolation of crystalline estrogenic compounds from extracts of these organs Table III lists the estrogen and the endocrine organ from which it was isolated The ovaries and placenta are generally regarded as the chief sources of estrogen in the organism The testes and adrenals appear to produce much smaller quantities although, in the stallion, testis tissue appears to be a prodigious producer of estrogen Thus, Levin (101) finds that certain specimens of stallion urine are the richest sources of α-estradiol to date and Beall (9) reports that the estrogenic content of horse testis is higher than that of any other endocrine organ

B Isolation of Estrogens from Sources Other Than Those of Elaboration

Pregnancy urine is a very rich source of estrogenic material The estrogens are present, for the most part, in the form of conjugates such as estrone sulfate[6] or estriol glucuronide, brief acid hydrolysis suffices to liberate the estrogen (Marrian in 37) The urine of nonpregnant women is a poor source as is also the urine of males with the notable exception of stallion urine (Zondek, 200, Levin, 101) The estrogen and its urinary source is indicated in Table IV Not all species elaborate estrogens of identical structure although estrone and estradiol are common to those species which have been studied Estriol appears to be characteristic of the human species, estrogens in which ring B is aromatic or partially saturated are found only in the mare

C Intermediary Metabolism of Estrogens

A comparison of the chemical structure of the various estrogens which have been obtained in exhaustive isolation studies (Tables III and IV) suggest metabolic interrelationships, some of which have been substantiated by experiment It has been fairly well established that the following reactions occur in the mammalian organism

$$\alpha\text{-estradiol} \rightleftharpoons \text{estrone} \rightarrow \text{estriol}$$
$$\Updownarrow$$
$$\beta\text{-estradiol}$$

This scheme is based on the isolation or detection of metabolites of the estrogen under study following its administration in massive doses to an experimental subject The results of such experiments are summarized in Table V There are differences among the species with respect to the course of estrogen metabolism For example, the formation of β-estra

[6] The preparation of estrone sulfate from estrone has been described by Butenandt and Hofsteter (27)

TABLE IV

ISOLATION OF ESTROGENS FROM URINARY SOURCES[a]

Estrogen	Source	Investigator
Ketonic estrogens		
Estrone	Pregnancy urine (human)	Doisy *et al* (50)
		Butenandt (21)
	Pregnancy urine (mare)	DeJongh *et al* (42)
	Male urine (human)	Dingemanse *et al* (44)
	Male urine (stallion)	Haussler (69)
		Deulofeu and Ferrari (43)
	Male urine (bull)	Marker (110)
	Castrate male urine (steer)	Marker (110)
Estrone sulfate	Pregnancy urine (mare)	Schachter and Marrian (154)
		Butenandt and Hofsteter (27)
Equilin	Pregnancy urine (mare)	Girard *et al* (62)
Hippulin	Pregnancy urine (mare)	Girard *et al* (62)
Equilenin	Pregnancy urine (mare)	Girard *et al* (61)
Nonketonic estrogens		
α-Estradiol	Pregnancy urine (human)	Smith *et al* (169)
	Pregnancy urine (mare)	Wintersteiner *et al* (199)
α-Estradiol	Male urine (stallion)	Levin (101)
β Estradiol	Pregnancy urine (mare)	Hirschmann and Wintersteiner (80)
Estriol	Pregnancy urine (human)	Marrian (119)
		Doisy *et al* (48)
Estriol glucuronide	Pregnancy urine (human)	Cohen and Marrian (36)
β Dihydroequilenin ("δ-follicular hormone")	Pregnancy urine (mare)	Wintersteiner *et al* (198)

[a] Exclusive of metabolism experiments

diol rather than α estradiol is favored in the rabbit when estrone (Stroud, 175, Pearlman and Pearlman, 132) or α-estradiol (Heard *et al*, 71, Fish and Dorfman, 59) is injected, whereas little or no β-estradiol can be detected in the urine of men following estrone administration (Pearlman and Pincus, 134) In human pregnancy urine little or no β-estradiol can be detected (Pearlman and Pearlman, 132), although α-estradiol (Smith *et al*, 169) has been isolated from this source Estriol has been isolated only from human source material but this substance (or one that closely resembles it in its physical properties and/or its biological action) may be formed in the guinea pig (Fish and Dorfman, 58), rabbit (Pincus and Zahl, 140 Pearlman and Pearlman, 132), monkey (Doisy *et al*, 49), dog (Longwell and McKee, 103 Pearlman *et al*, 131), and rat (Schiller and Pincus,

TABLE V

ESTROGEN METABOLISM EXPERIMENTS

Estrogen metabolites[a]		Subject	Investigator
Isolated	Indicated[b]		
Administered α-estradiol			
Estrone, β-estradiol		Rabbits female (with and without simultaneous progesterone administration)	Heard *et al* (71)
		Rabbits ovariect and hysterect	Fish and Dorfman (59)
Estrone	Strong phenolic estrogen (estriol?)	Guinea pigs both sexes ovariect	Fish and Dorfman (58)
Estrone		A man	Heard and Hoffman (73)
	Ketonic estrogen (estrone?)	Monkeys female ovariect ovariect hysterect	Westerfeld and Doisy (189)
	Estrone	Rabbits female (ovaries essential to conversion)	Pincus and Zahl (140)
	Estriol	Rabbits female (functional uterus essential to conversion)	
	Ketonic estrogen (estrone?)	Dogs both sexes	Dingemanse and Tyzlowitz (45)
None		Pregnant monkeys	Marker and Hartman (111)
	In bile estrone estriol	Dogs	Pearlman *et al* (131)
	Liver perfusate estrone estriol	Rat liver	Schiller and Pincus (155)
	Estrone Estriol	Humans	
Administered estrone			
β Estradiol Estriol	α-Estradiol	Rabbits Men	Stroud (175) Pearlman and Pincus (133)

[a] Excreted in urine except where otherwise noted

[b] Indicated indirectly by comparison of the chemical and physical properties of the substances responsible for the estrogenic activity of postinjection urine extracts with those of pure crystalline hormones

TABLE V (*Continued*)

Estrogen metabolites[a]		Subject	Investigator
Isolated	Indicated[b]		
	Nonketonic estrogens	Monkeys female ovariect, ovariect and hysterect	Westerfeld and Doisy (189)
	Estriol	Rabbit female (functional uterus essential to conversion)	Pincus and Zahl (140)
	Estradiol Estriol	Women (simultaneous progesterone administration)	Smith and Smith (168)
	Nonketonic estrogens	Dogs both sexes	Dingemanse and Tyzlowitz (45)
	Nonketonic estrogens	Dogs sex?	Longwell and McKee (103)
	In bile "weak" and "strong" phenolic estrogens		
	Estradiol Estriol	Men and women	Pincus and Pearlman (138)
	α-Estradiol	Rat and rabbit liver, uterus etc, slices	Heller (76)
	β Estradiol Estriol	Rabbits female	Pearlman and Pearlman (132)
	Estradiol Estriol	Rats partially hepatectomized	Schiller and Pincus (156)
	α Estradiol	Rabbit (pregnant) endometrium	Szego and Samuels (180)
Administered estriol			
	Estriol no estrone, nor estradiol	Monkey female, normal intact, ovariect and hysterect	Doisy *et al* (49)
		Rabbits female	Pincus and Zahl (140)
	Estriol no estrone nor estradiol	Men	Schiller and Pincus (155)
Administered β estradiol			
	Estrone α-estradiol?, estriol	Monkey female, ovariect and hysterect	Doisy *et al* (49)

155,156), identification of this estrogen metabolite by isolation of a crystalline product would be desirable Marrian (120) suggests that estriol may have some specific but unknown function during pregnancy

Studies of the metabolism of equilin or equilenin have not been reported and there is only conjecture as to the relative position of these substances in the scheme of estrogen metabolism Fieser (56) suggests that compounds of the equilenin group may represent successive stages in the dehydrogenation of estrone In the laboratory, estrone can be dehydrogenated to give a stereoisomer of equilenin (see Section I, D, 6) Equilin readily undergoes dehydrogenation to yield equilenin (see Section I, D, 3), a process which may also occur *in vivo*

D Is Estrogen Metabolism Confined to Organs of Sex Hormone Production and Stimulation?

As far as is known, very little or no estrogenic material is produced in organs other than the ovaries, testes, placenta, and adrenals, possibly the pituitary gland produces some estrogen but this has not been estab lished It is quite logical to assume that the end organs of estrogen stimulation, such as the uterus, transform the estrogens into other prod ucts in a process intimately linked with the biological utilization of these hormones Indeed, *in vitro* experiments indicate that uterine tissue will modify the structure of the estrogen molecule Thus, Heller (76) incubated estrone with rat and rabbit uterine tissue and observed an increase in estrogenic potency Szego and Samuels (180) demonstrated an almost complete conversion of estrone into α-estradiol following incubation of estrone with the endometrium of the pregnant rabbit, however, this change could not be effected by the endometrium obtained from nonpregnant bovine or from a pregnant woman Such experiments do not necessarily mean that the intermediary metabolism of estrone is confined to the uterus As a matter of fact, the bulk of the evidence indicates that the uterus (and ovaries) (see Table V) are not essential to those chemical transformations which the estrogens are known to undergo, but then it must be borne in mind that our knowledge of estrogen metabolism is meager Marker (109) made the interesting suggestion that the regulation of the menstrual cycle is controlled by estrogenic substances by a process involving the reduction of these substances to the biologically inactive estranediols (LXX), which he had isolated from nonpregnancy urine but not from pregnancy urine It has not been proved that the biological effect is dependent on this chemical change nor has it even been established that the estrogens are reduced in the organism to estranediol It seems that our knowledge of the biochemical mechanisms whereby estrogens exert their biological effects is practically nil

There is considerable evidence that the liver plays a major role in the transformation of the estrogens into products lacking biological activity (see Section II, F) The liver and certain other organs can also convert the estrogens into other products possessing more or less estrogenic potency Heller (76) incubated estrone with liver slices poisoned with cyanide and noted the formation of a product of increased estrogenic potency, presumably α-estradiol The addition of cyanide inhibits the inactivating system of the liver, the *in vitro* conversion of estrone into a substance possessing greater biological activity cannot be demonstrated otherwise In the perfusion experiments of Schiller and Pincus (155), rat liver appears to convert α-estradiol into estrone and estriol or into estrogenic substances which are very similar in physical and chemical properties If the estrogenic substances found in bile are assumed to be formed by metabolic processes occurring in the liver, estrogen metabolism studies on bile fistula dogs may be of special interest in this regard Longwell and McKee (103) found that, if dogs were injected with estrone, a nonketonic estrogen appeared in bile This estrogen is extractable from benzene with sodium carbonate solution Pearlman *et al* (131) detected a similar estrogen (estriol?) in the bile following the intravenous injection of α-estradiol, a ketonic estrogen, presumably estrone, was the major estrogenic metabolite detected According to Szego and Roberts (147,178), the liver is essential for estrogenic activity as measured by the uterine water response in the adult, partially hepatectomized or eviscerated rat Two mechanisms are envisaged which may explain this phenomenon (*1*) the liver inactivates estrogens and is also essential for the chemical "activation" of estrogens and (*2*) the liver may furnish some metabolite essential for the uterine response

E Isolation of Nonphenolic Steroids Structurally Related to Estrogens Are These Metabolically Related?

The isolation of certain nonphenolic steroids from urinary sources (see Table VI) has provoked speculation as to whether these substances may not be derived *in vivo* from the estrogens since there is a close structural relationship In the laboratory, the conversion of equilenin into $\Delta^{5,7,9}$-estratrienol 3-one-17 has been realized (see Section I, D, 2), the latter substance was isolated by Heard and Hoffman (72) from mare pregnancy urine Whether the organism can effect a similar conversion is not known The estranediols isolated by Marker *et al* (114,117) from human nonpregnancy urine may have arisen from estrone *in vivo* since catalytic hydrogenation of estrone yields substances of this type Doisy *et al* (49) have pointed out that dehydrogenation of some saturated ring

TABLE VI

ISOLATION OF NONPHENOLIC STEROIDS[a]

Steroid	Urinary source	Investigator
Estranediol A and B (LXX)	Human pregnancy	Marker *et al* (116 117)
$\Delta^{5,7,9}$ Estratr enol 3-one-17 (see LXXII)	Pregnant mare	Heard and Hoffman (72)
3-Desoxyequilenin (LXXIXa)	Pregnant mare	Prelog and Fuhrer (141)
11 Keto-3-desoxy-equilenin (after CrO_3 oxidation) (LXXIXb)	Pregnant mare	Marker and Rohrmann (114 115b)

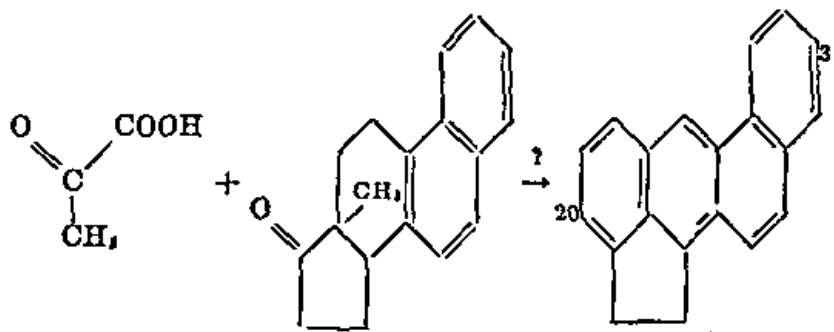
[a] Of close structural relationship to estrogens

structures is effected by mammals but that the reduction of aromatic rings is of less frequent occurrence It was suggested that these reduction products of estrogens may be anabolites rather than catabolites of the estrogens In this connection the experiments of Bernhard and Caflisch Weill (10) are pertinent They found that hexahydrobenzoic acid labeled with deuterium is dehydrogenated in the dog to benzoic acid, the aromatization of the cyclohexane ring readily occurs *in vivo* It may be that aromatization of the steroids occurs in the organism

The recent isolation by Prelog and Führer (141) of 3 desoxyequilenin (LXXIXa) from pregnant mares urine is exceedingly interesting Marker and Rohrmann (115b) had previously suspected the presence of this substance in pregnant mares urine since 11 keto-3-desoxyequilenin (LXXIXb) was isolated from the neutral fraction after chromic acid oxidation, similar oxidation of equilenin acetate will result in the introduction of a keto group at C_{11} (see Section I D 4 b) There is no basis of chemical analogy to support the hypothesis that desoxyequilenin arises directly from equilenin in the organism It is true that catalytic reduction of the estrogens in an acid medium will result in the elimination of the hydroxyl group at position 3 but saturation of ring A occurs concomitantly On the other hand it is conceivable that 3-desoxyequilenin arises *in vivo* from $\Delta^{5,7,9}$-estratrienol-3 one 17 (LXXII) by a process involving dehydration in ring A followed by aromatization since on chemical grounds at least this process is easily visualized (By virtue of the same reasoning 3 desoxyequilenin may possibly have arisen as an artifact in the course of isolation, the formation of artifacts has been a sore point with the student of steroid metabolism in the past—for examples see "Artifacts in the study of the intermediary metabolism of androgens in 139) It is not unlikely that 3 desoxyequilenin is converted to equilenin in view of the demonstrated hydroxylation *in vivo* of certain

naturally occurring aromatic substances, *e g*, the conversion of phenylalanine (labeled with deuterium) to tyrosine (Moss and Schoenheimer, 124), the conversion of stilbene to 4,4′-dihydroxystilbene in the rabbit is another example (Stroud, 176)

HYPOTHETICAL CONVERSION *in vivo* OF THE ESTROGENS[a] TO CARCINOGENS[b]



Pyruvic acid — 3-Desoxyequilenin (LXXIXa) (formula inverted) — Cholanthrene

⇓?

equilenin (XIV)

⇓?

Estrone (XIX)

[a] LXXIXa XIV XIX have actually been isolated from pregnancy urine

[b] Adapted from Fieser (55)

The fact that a highly aromatic steroid such as 3 desoxyequilenin has been isolated from natural sources is highly significant in connection with that theory of carcinogenesis which postulates the formation of polynuclear substances akin to synthetic substances of demonstrated carcinogenic activity *e g* 20-methylcholanthrene, by a "faulty" metabolism of the steroids (see Fieser, 56), 20-methylcholanthrene can be prepared synthetically from the innocuous bile acids and also from cholesterol More recently Fieser (in 55) suggested that equilenin (or estrone) might undergo condensation with pyruvic acid *in vivo*, resulting eventually in the formation of 3 hydroxycholanthrene (The latter substance has not been prepared synthetically) In view of the fact that 3 hydroxy-20-methylcholanthrene, which has been prepared synthetically, is lacking in potency as a carcinogen (probably due to the presence of the 3 hydroxyl group) Fieser does not feel too sanguine with regard to the hypothetical formation of cholanthrene or its derivatives, it was difficult to visualize how the phenolic hydroxyl group could be eliminated *in vivo* However, the recent isolation of 3 desoxyequilenin clearly removes, at any rate, this objection although to be sure it is not known whether 3 desoxyequilenin actually arises from equilenin or from some other substance 3 Desoxyequilenin may therefore conceivably give rise to cholanthrene, a substance which has been synthesized and which is known to be very

potent as a carcinogen The fact that only 3 6 μg of 3-desoxyequilenin is present per liter of pregnant mares' urine (calculated from the isolation data) is in itself interesting, for it indicates that significant steroid metabolites, *e g*, carcinogens or procarcinogens, may be present in biologic material in amounts that would ordinarily escape detection

F Role of Liver in Estrogen Inactivation

1 *In Vitro Studies*

a Incubation Experiments Zondek (201) first demonstrated a loss of estrogenic potency on incubation of estrogens with liver pulp, acid hydrolysis did not restore the estrogenic activity Since the inactivating property of the liver is destroyed by heating and since cell-free extracts retain their inactivating capacity, Zondek concluded that the estrogen inactivation is probably an enzymic process, the enzyme responsible was designated an "estrinase ' The observations of Zondek have been confirmed by Engel and Rosenberg (54), the latter authors succeeded in obtaining aqueous extracts from beef liver which are capable of rapidly inactivating the native and synthetic estrogens Zondek and Sklow (203) demonstrated that the reticuloendothelial cells of the liver play no part in the process of inactivation, the liver cell is believed to contain "estrinase "

In the experiments by Heller *et al* (76–78), α-estradiol was incubated with liver slices from the rat and rabbit, complete inactivation of the estrogen was observed and the estrogenic activity was not restored by acid hydrolysis Partial inactivation was effected by kidney slices, incubation with heart, lung, spleen, uterine, or placenta tissue did not decrease the estrogenic potency Estrone was completely inactivated by rabbit liver slices and partially by rabbit kidney and rat liver Estriol was only partially inactivated by rat liver and kidney and also by rabbit liver It appears then that there are differences in the rate of inactivation of the various estrogens and that there are also differences among species in the rate of inactivation of the same estrogen *In vitro* experiments by other workers further substantiate the latter conclusion Twombly and Taylor (183) showed that human liver tissue inactivates α estradiol less rapidly than does liver tissue from mice and rats In the experiments of Samuels and McCauley (153) the rate of estrogen inactivation was lowest when human liver mince was employed, inactivation was most rapid following incubation with liver mince obtained from the rat and mouse, other species studied were the rabbit and dog

b Perfusion Experiments Israel *et al* (94) perfused the heart-lung system of the dog with estrone, no inactivation was observed On the

other hand, when a heart-liver lung preparation was substituted, rapid inactivation ensued, histologic examination of the liver at the termination of the experiment appeared to indicate that the functional state of the liver had been maintained Schiller and Pincus (155) perfused rat liver with α-estradiol They observed some inactivation which they suggested might be accounted for by conversion of α-estradiol to the less potent estrogens, estrone and estriol If small amounts of α estradiol were perfused, complete inactivation resulted in the course of five hours, acid hydrolysis of the perfusate did not restore the activity On the other hand, perfusion of rat heart with α estradiol resulted in no loss of activity

2 *In Vivo Studies*

a Estrogen Experimentally Diverted into Hepatic Portal Circulation Golden and Sevringhaus (65) transplanted the ovaries of the rat to the mesentery and to the axillae, estrus did not occur in animals with the ovaries in the portal circulation but estrus did occur in those animals with transplants in the axillae Biskind and Mark (13,14) implanted pellets of estrogen in the spleen of castrated rats They observed no estrogenic effect as long as the spleen remained connected with the hepatic portal circulation When the spleen was transplanted so that its venous blood flowed directly into the systemic circulation, the estrogenic effect of the implanted hormone became apparent Biskind and Meyer (15) implanted estrone pellets in the spleen of male rabbits, degeneration of the testicles did not result as is the case when estrone is implanted subcutaneously These observations were confirmed by Segaloff *et al* (160 162), who carried out experiments of a similar nature

b Liver Damage In animals with liver damage induced by hepatotoxic agents or by dietary factors, there is an increased sensitivity of the end organs to endogenous and exogenous estrogen Thus, Talbot (181) noticed a definite increase in uterine weight in nonovariectomized rats which had received carbon tetrachloride Pincus and Martin (137) observed a marked increase in vaginal response on estrogen administration to ovariectomized rats which had received carbon tetrachloride by gavage The role of the nutritional state of the organism in estrogen metabolism is discussed below (Section II, G) It is of interest that Glass *et al* (63) noticed that male patients with cirrhosis of the liver displayed gynecomastia and testicular atrophy, the estrogen in the urine was largely excreted in unconjugated form

c Partial Hepatectomy Schiller and Pincus (156) studied the metabolism of estrone in normal and partially hepatectomized rats, there was an increased excretion of estrogen in the urine in the latter group of

animals Segaloff (161) observed that partial hepatectomy in spayed female rats reduces the amount of α-estradiol required to produce vaginal estrus when the estrogen is injected subcutaneously or intrasplenically Engel and Navratil (53) observed that, in the hepatectomized frog, estrone is inactivated after 48 hours, they concluded that the liver is not the only organ in which inactivation occurs This conclusion does not, of course, necessarily apply to mammals It is interesting that Selye (164) found the anesthetic effect of the steroids to be increased by partial extirpation of the liver To be sure, this is a totally different type of biological response and is properly classified by Selye as pharmacological

G Effect of Nutritional State on Estrogen Metabolism[6]

Biskind and Shelesnyak (17) claim to have demonstrated a relationship between vitamin B complex deficiency and the capacity of the liver to inactivate estrogens Following castration of adult female rats and transplantation of one ovary to the spleen, the animals developed an anestrous condition When the rats were placed on a vitamin-B-complex free diet most of these animals went into a state of estrus, there were no visible organic lesions in the liver In a subsequent study by Biskind and Biskind (16), pellets of estrone were implanted in the spleen of adult castrated female rats On a normal diet the rats were anestrous but on a vitamin B-complex-deficient diet, a protracted state of estrus developed Addition of brewers' yeast to the vitamin-deficient diet effected a return to the anestrous state The observations of Biskind and co-workers were confirmed by Segaloff and Segaloff (163) They noted an increased vaginal response to estrogen in spayed rats on a B-complex-deficient diet (as compared to spayed rats on a normal controlled diet), the estrogen was injected intrasplenically or subcutaneously Addition of choline chloride, pyridoxine or calcium pantothenate to the B-complex-deficient diet failed to decrease the vaginal response of these rats to the level of the rats on the normal diet, whereas addition of thiamin hydrochloride and riboflavin effected this result The authors state that the effect of thiamin hydrochloride on the vaginal response to estrogen administration is independent of its effect on the appetite of the experimental animals Singher *et al* (167) made *in vitro* studies bearing on the relationship of vitamin deficiency to the capacity of the liver to inactivate estrogens They observed that slices of liver removed from rats on a riboflavin and thiamin deficient diet were unable to inactivate estradiol, whereas liver slices from rats on a normal diet were capable of doing so Furthermore, the loss of inactivating ability paralleled the decrease in the riboflavin and thiamin content of the liver Pyridoxine,

[6] See recent reviews by Hertz and also by Biskind in (184)

pantothenic acid, biotin, and vitamin A had no effect on estrogen inactivation under the same experimental conditions

Shipley and Gyorgy (166) performed experiments similar to those of Biskind and Biskind (16) with the essential difference that the rats which were utilized had hepatic injury deliberately induced by feeding a high fat, low protein diet It will be recalled that in Biskind s experiments no hepatic lesions were visible Nonetheless the results with respect to vaginal response were about the same A curative effect was observed when large amounts of yeast were included in the diet Gyorgy (68) showed subsequently that the addition of lipotropic factors such as methionine or protein digest to the diet likewise exerted a curative effect, but a fivefold increase in the basal vitamin B supplement did not affect the results As to whether the accumulation of fat *per se* or destruction of hepatic cells or both is the responsible factor in the increased vaginal response to estrogen in Gyorgy s experiments it appears from the work of Szego and Barnes (177) that the dietary accumulation of fat in the liver is not the responsible factor

Recently the conclusions drawn from most of the foregoing studies were questioned by Drill and Pfeiffer (51) They point out that in these studies paired inanition controls were not used To be sure the observations of Biskind and Biskind (16) were readily confirmed by Drill and Pfeiffer but paired inanition control rats which were limited to the same amount of food consumed by the vitamin deficient animals (but receiving the B vitamin) also showed vaginal cornification The authors (51) conclude therefore that the effect of acute vitamin B complex deficiency appears to be due to the concomitant inanition, addition of methionine to the diet did not influence the results

Koref and Engel (99) observed that folic acid will inhibit to a limited extent the estrogenic activity of estrone after the two substances are incubated *in vitro* Although folic acid is present in liver, the authors suggest that folic acid may not be the substance responsible for the vigorous estrogen inactivating potency of the liver *in vitro*

H Enzymic Inactivation of Estrogens

It is generally believed that catabolism of the estrogens will result in the formation of products lacking in biological activity, this process has been loosely referred to as "inactivation" Nothing is known concerning the nature of such products These should not be confused with the conjugated forms of the estrogens such as estrone sulfate and estriol glucuronide the latter are weakly estrogenic and are readily detected by subjecting the conjugates to acid hydrolysis which liberates the estrogen

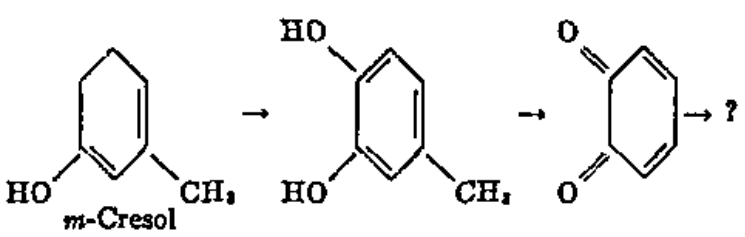
The source and nature of the enzymes or enzyme systems which inactivate estrogens are discussed below

1 *Liver*

Zondek (201,202) has been successful in obtaining a cell free extract of liver which will destroy the biological potency of estrogens[7], the presence of an enzyme, "estrinase," has been postulated Engel and Rosenberg (54) have also succeeded in preparing similar aqueous extracts from beef liver, such extracts may be treated with organic solvents to precipitate the inactivating factor in the form of a powder It would be highly desirable to obtain a highly potent preparation of this character so that the nature of the inactivating process and the structure of the products of inactivation might be determined Heller (76) believes an oxidase system is responsible for the inactivation of estrogens by liver slices but the presence of such a system could not be demonstrated by Graubard and Pincus (66)

2 *Plants*

Westerfeld (187) obtained from mushrooms a tyrosinase extract which converts tyrosine to melanin and is also capable of inactivating estrone The products formed from estrone may be similar to the products described by Raper (144) when tyrosinase is permitted to act on *m* cresol



The oxidative nature of the enzymic inactivation of estrogens has been demonstrated by Graubard and Pincus (66) They found that potato tyrosinase transforms the estrogens into colored products with the uptake of three to four atoms of oxygen, the hormones appeared to be completely inactivated Laccase action will result in the uptake of about one atom of oxygen per molecule of hormone with a loss of 90% of the estrogenic activity Mushroom tyrosinase does not appreciably oxidize estrogens nor does it affect their activity Zondek and Sklow (203) described the preparation of an estrone-inactivating enzyme from potatoes, which they believe to be neither tyrosinase nor a laccase These authors (cited in 203) also found that beet root and potato juice contain a tyrosinase capable of inactivating estrone Hyacinth roots and cauli-

[7] Synthetic estrogens *e g*, stilbestrol may also be inactivated by rat liver pulp (Zondek *et al*, 206)

flower juice will destroy estrone although they do not contain a melanin-forming tyrosinase However, the melanin-forming tyrosinase from mealworm larvae does not inactivate estrone Recently Zondek and Sulman (204) examined 32 strains of nonpathogenic bacteria and found that *Proteus X* kingsbury and *Bacillus mesentericus* will inactivate estrone[8], the presence of a melanin forming tyrosinase could not be detected Examination of 29 pathogenic strains of bacteria did not reveal any strain capable of estrogen inactivation

While it is true that the inactivating factors present in the plant kingdom have not been demonstrated in the animal organism, still much can be learned by the type of studies described above, similar substances might be formed in the mammalian organism although perhaps by a different mechanism [9] It would be very interesting to isolate the end products of estrogen inactivation and to determine their structure Probably these substances are exceedingly labile and may defy isolation from such sources as urine

I Estrogen in Bile and Blood

1 *Excretion of Estrogens in Bile an Enterohepatic Circulation of Estrogens?*

A very high excretion in the bile of dogs and humans of estrogen of both endogenous and exogenous origin has been observed by Cantarow and co-workers (32–35) For example, as much as 90–95% of the biological activity was recovered in the bile of dogs following the intravenous injection of either estrone or α-estradiol (35) Although it had been known that bile contains estrogenic material,[10] (literature cited by Cantarow *et al*, 35), it had not been demonstrated until recently that the quantities of estrogen excreted in the bile are large by comparison with the amounts to be found in the peripheral circulation or in the nonendocrine organs of the body These findings led Cantarow *et al* to postulate an enterohepatic circulation of the estrogens similar to that of the bile acids These investigators (35) do not deny that the liver can inactivate estrogens but they doubt that the liver does so *in vivo* as rapidly as has been supposed The experiments *in vitro* indicate that the liver rapidly inactivates estrogen (see Section II, F, 1), but the experiments *in vivo* performed by Cantarow *et al* do not support this conclusion *In vitro*

[8] Estrogens may undergo metabolic change by the intestinal flora, estrogens are known to be excreted into the gut (see Section I, I, 1)

[9] Tyrosinase like ' activity has been detected in the hide of the rat by Spoor and Ralli (174)

[10] Early observations by Gsell-Busse (67)

studies cannot be strictly compared to *in vivo* studies, the results may perhaps be the same qualitatively but may differ quantitatively Those *in vivo* studies which purport to demonstrate a rapid inactivation of estrogens (see Section II, F, 2) can be interpreted quite differently on the basis of an enterohepatic circulation of estrogens Thus in those experiments in which estrogen is directed into the hepatic circulation (see Section II, F, 2, a), a diminished biological response is to be anticipated since, under the experimental conditions, little estrogenic substance can enter the systemic circulation whereas, under physiological conditions, estrogen is secreted into the systemic circulation This interpretation appears to be borne out by the demonstration of considerable estrogenic activity in the bile collected from a dog in which a pellet of α-estradiol was implanted in the spleen (34)

In those studies in which the liver is damaged (see Section II, F, 2, b) or in which the experimental animals are maintained in a state of inanition by limiting food consumption (see Section II, G), the increased estrogenic response to estrogen may be the consequence of a decreased flow of bile or a diminished excretion of estrogen in the bile, or both [11] It is more difficult to explain why slices of liver from rats on a B complex-deficient diet should show a diminished capacity to inactivate estrogens on incubation It is noteworthy that Cantarow *et al* (32) found no difference in the rate of inactivation of estrogen by liver brei obtained from rats poisoned with carbon tetrachloride, a hepatoxic agent, as compared with liver brei obtained from healthy animals

The excretion of large amounts of estrogen in the bile was not observed by Longwell and McKee (103), who recovered only 1 3 to 8 0% of the biological activity in the bile following the subcutaneous injection of estrone in dogs Thus, there is lack of agreement between these laboratories with respect to the level of excretion of exogenous estrogen in the bile Cantarow *et al* (35) stress the necessity of ensuring a satisfactory state of efficient liver function in bile fistula dogs since otherwise relatively insignificant hepatic functional defects may have a profound influence on steroid hormone metabolism It has become increasingly apparent in recent years that nutritional factors must be reckoned with in evaluating the level of estrogen metabolism in the organism

As Longwell and McKee (103) have pointed out, the liver appears to be intimately linked with the metabolism of estrogens and the bile seems to be a likely source of material with which to study these reactions These investigators found that the bile contains estrogenic substances other than the estrone administered to bile fistula dogs Pearlman *et al*

[11] A vasoconstrictor effect on intrahepatic circulation in the rat results from brief inhalation of carbon tetrachloride vapors (Wakim and Mann, 185)

(131) similarly demonstrated the presence of metabolites of estradiol in the bile following the administration of this estrogenic substance

Appreciable amounts of estrogen have been detected in the feces of the pregnant cow (Levin, 100), the major portion of the estrogenic activity is found in the weakly phenolic, nonketonic fraction and it may therefore be presumed that estradiol is present The excretion of estrogens in the feces may be due to the fact that estrogens are normally excreted in the bile and may escape, in part, absorption in the gut, it is also possible that estrogen is excreted into the gut directly from the blood stream A fecal excretion of estrogens in other species has been noted by other investigators (literature reviewed by Levin 100) The intestinal flora may play a role in effecting chemical changes in the estrogen excreted since it is well established that the estrogens (and other steroids) undergo phtyochemical change (for a review of pertinent literature, see 139)

2 *Nature of Estrogen Circulating in Blood*

The estrogen in the blood is maintained at a relatively low level even under those physiological conditions when estrogen production is maximal, *e g*, during pregnancy (Szego and Roberts, 179) Intravenously injected estrogen disappears rapidly from the blood stream (Cantarow *et al*, 32) Whatever the explanation for these phenomena (see discussion above) it is evident that the concentration of estrogen arriving at the target organs of estrogen stimulation must be at a low level It is of obvious importance to know the nature of this "transport-estrogen"

Pursuit of this problem has been handicapped by the practical difficulty involved in obtaining from the blood amounts of estrogenic material adequate for isolation study Nevertheless, some information of an indirect nature has been obtained Haussler (70) reported that variable amounts of estrogen are associated with the proteins in mare serum Muhlbock (125) observed that about 30–50% of the estrogenic substances in the blood of pregnant mares is in a combined form from which it is released on acid hydrolysis, in pregnant women, 50–75% of the blood estrogens are in the free form (Muhlbock, 126) Rakoff *et al* (143) found that the concentration of estrogens in the serum of pregnant women is identical with that in whole blood, as much as 50% of the total estrogenic material of the serum may be present in a combined or conjugated form Szego and Roberts (179) have made extensive studies of blood estrogen They observed that the estrogen content is uniformly low in the blood or serum obtained from pregnant women, normal and pregnant cows, and normal and gonadotrophin injected rabbits Protein free acetone extracts of blood contain about one third the total estrogenic content of the blood, the estrogen in these extracts is almost entirely

present in a conjugated form since it can be extracted with ether only after acid hydrolysis The rest of the estrogenic material is associated with the blood proteins in a form liberated by weak alkaline hydrolysis The blood estrogens will pass through a collodion membrane and the dialyzate so obtained contains all of the estrogenic material originally present in the blood It is interesting that they were able to dialyze aqueous solutions of the crystalline estrogens The authors conclude that there is an equilibrium in the blood between estrogen and protein and that dialysis results in the progressive dissociation of the estrogen protein complex Roberts and Szego (146) utilized human plasma material recently made available by the Cohn method of fractionation and found that the lipoprotein fraction III O (α β globulin fraction) contains most of the estrogenic material All the estrogenic activity of this fraction may be found in the dialyzate the estrogen is present in an esterified form from which it can be released by brief acid hydrolysis The estrogenic material thus obtained resembles estriol in its physical properties The authors suggest that the conjugated form of the estrogen may be that of a glucuronide It is also suggested that the liver may be the site of formation or combination of the estrogen protein complex The reports of Roberts and Szego leave the impression that practically all the estrogenic material in blood or plasma is present in a conjugated form not extractable by ether whereas other workers in this field have indicated the presence in blood of considerable amounts of estrogen existing in a form which can be readily extracted by ether *i e*, the free form

J Some Conjectures Regarding Metabolism of Estrogens

Zondek and Sulman secured indirect evidence (205) on the basis of experiments with gonadotrophic and antigonadotrophic factors for the formation of a "pro-estrogen" in the ovaries of infantile female rats during the eighteen hour period following the injection of chorionic gonadotrophin They also cite the work of Freed and Soskin (60) who stimulated rat ovaries with chorionic gonadotrophin and obtained evidence for the formation of two estrogens one in the theca and the other in the granulosa The theca estrogen was found to be incomplete in its action since it did not induce endometrial proliferation The granulosa estrogen was complete in its action and resembled estrone in this respect It would be very interesting to determine the chemical structure of these substances but an attempt to isolate these from ovarian extracts would probably not be practical

The androgens may possibly serve as precursors of the estrogens in

the organism, as was first suggested by Butenandt and Kudszus in 1935 (28) These investigators observed that androstenedione elicited an appreciable estrogenic response in infantile female rodents, they considered the possible conversion of androstenedione into estrogenic substances in the ovary It is of interest in this connection that some evidence has been obtained which indicates that the ovaries secrete androgenic substances, but the nature of these substances is not known (literature cited in 139)

Paschkis *et al* (129,130) attempted to demonstrate a biological transformation of androgens into estrogens They detected the excretion of estrogenic material in the bile and urine of female dogs following the administration of androgens Since the amounts of estrogen excreted were very small, it is possible that the ovaries may have been stimulated to secrete additional estrogen, but according to these authors, the fact that estrogen was present in the urine following the injection of testosterone in normal and castrated male dogs (129) militates against this assumption The reviewer is of the opinion that no conclusion can be arrived at with respect to the ovarian factor since castrated female dogs were not utilized There is also the possibility that the adrenal glands of either sex may have been stimulated by the androgens injected The adrenal factor is not eliminated by the observation, in a subsequent experiment that very small quantities of estrogen are excreted by a female adrenalectomized dog since this animal was not castrated Paschkis *et al* (129) finally conclude that the conversion of androgen into estrogen seems to be more a likely interpretation but is not yet definitely proved in none of these experiments was an excretion of estrogen observed prior to androgen administration It is noteworthy that certain androgenic substances, especially Δ^5-androstenediol-3(β),17(α), possess some estrogenic activity (Butenandt, 23) Whether Δ^5-androstenediol-3(β),17(α) is actually converted *in vivo* to a substance identical with or closely resembling the native estrogens in structure is not known, the estrogenic activity of these androgenic substances may be due to their *intrinsic structure It might be interesting to see whether the excretion* of estrogenic material is actually higher in dogs injected with Δ^5 androstenediol-3(β) 17(α) than in dogs similarly treated with testosterone, it might also be of interest to carry out such experiments with gonadectomized adrenalectomized animals In recent years, a chemical basis of analogy has been established for the biological conversion of androstenediol into α-estradiol (see Section I, B, 2) Cholesterol may be the ultimate source of estrogens in the organism, the metabolic route may perhaps be by way of dehydroisoandrosterone (Butenandt and Kudszus, 28, Fieser, 56) Rondoni *et al* (150) claim to have detected small

amounts of estrogenic material in liver following incubation with cholesterol over a period of several weeks Since incubation of estrogens with liver is known to result in inactivation, the extent of conversion of cholesterol to estrogen may have been considerably higher The chemical nature of the estrogenic material obtained in the experiments of Rondoni *et al* (150) was not established The results obtained are not clear-cut and imply that organs other than those of internal secretion may serve as sources of estrogen elaboration, an inference that has not been substantiated to date

A method devised by Emmens (52) may be exceedingly useful for the detection of metabolic precursors of the estrogens According to this author, pro-estrogens may be differentiated from true estrogens by determining the relative effective dose required to elicit a vaginal response when the substance tested is administered by the systemic (S) and local (L) routes Compounds which have a low S/L ratio are regarded as pro-estrogens on the assumption that such compounds, in order to exert a maximal biological response, must enter the systemic circulation to undergo modification by some organ other than the vagina Of the substances thus far tested and found to be pro-estrogens, none are steroid in character with two notable exceptions one of these is androstenediol (see discussion above) Useful "leads" as to the nature of estrogen precursors might be gained by a more extensive application of the Emmens method

Ample ground for speculation in the field of estrogen metabolism is afforded by our expanding knowledge of the chemistry of the estrogens For example, the recent findings of Miescher and co workers (see Section I, C) that certain acids prepared by total synthesis or by partial synthesis from the native estrogens are more active than α-estradiol raises the question in the minds of these investigators of whether these substances (or perhaps similar ones) may be formed *in vivo* The student of estrogen metabolism, having been alerted by work of this character, may be encouraged to make a more exhaustive study of the acid fractions obtained from extracts of biological material Bioassay by the vaginal smear technique should be of great aid in this instance in following the course of isolation, but it would be useless in the detection of a product such as marrianolic acid which is lacking in estrogenic activity Lactones may arise in the course of estrogen catabolism, compounds of this character have been prepared by treating the native estrogen with chromic acid, permanganate, or hydrogen peroxide (see Section I, C, and I, D) Reactions with hydrogen peroxide are believed to resemble reactions which occur *in vivo* and it is for this reason that the weakly estrogenic lactone, which Westerfeld (188) obtained by treating estrone with hydrogen peroxide,

may be of special significance According to Smith (170,171), this lactone is considerably more potent than estrone in stimulating the pituitary, a remarkable finding indicative of the existence in nature of a novel category of estrogens A differential method of assay based on the pituitary and vaginal response may yet reveal the existence of metabolites identical with or similar to Westerfeld's lactone Compounds such as 16-ketoestrone or 16 hydroxyestrone, which are hypothetical intermediates in the biological conversion of estrone to estriol, and also 7-ketoestrone would probably be decomposed by the strong alkali ordinarily employed in extracting estrogens, 7-ketoestrone is readily destroyed by treatment with aqueous sodium carbonate at room temperature (Pearlman and Wintersteiner, 135) It is conceivable that the marked increase in biological potency observed by Smith and Smith (172), as a result of the addition of zinc dust prior to the acid hydrolysis of urines, may be traced to the reduction of alkali labile estrogenic material, this increase in estrogenic activity cannot be due entirely to the conversion of estrone to estradiol, according to Smith and Smith

In retrospect, it can be said that our present knowledge of estrogen metabolism is fragmentary but the future appears to be very promising It is becoming increasingly apparent that compounds which can be prepared in the laboratory from the estrogenic hormones can, in many instances, be isolated from natural sources as well Thus our knowledge of the chemistry of the estrogens quickens our efforts in solving problems of estrogen metabolism The application of the new tool of isotope labeling may prove to be as highly illuminating in this field as has been its recent application in studying the related fields of steroid metabolism (for example see Bloch *et al*, 18) Although much has been, and probably will be learned by the classical approach to metabolism investigation deficiencies inherent in such methods render the study of certain problems difficult if not impossible, as for example, the hypothetical conversion of cholesterol to estrogen

REFERENCES

1 Anner G Heer J and Miescher K *Helv Chim Acta* **29**, 1071 (1946)
2 Anner G and Miescher K *ibid* **29** 586 (1946)
3 Bachmann W E Cole, W and Wilds A L *J Am Chem Soc* **61**, 974 (1939), *ibid* **62**, 824 (1940)
4 Bachmann W E Gregg R A and Pratt, E F *ibid* **65**, 2314 (1943)
5 Bachmann W E and Holmes, D W *ibid* **62**, 2750 (1940) *ibid* **63**, 595 (1940)
6 Bachmann W E Kushner S, and Stevenson A C *ibid* **64**, 974 (1942)
7 Bachmann W E and Wilds A L *ibid* **62**, 2084 (1940)
8 Beall D *Nature* **144** 76 (1939)

9 Beall, D *Biochem J.* **34**, 1293 (1940)
10 Bernhard, K , and Caflisch-Weill, H *Helv Chim Acta* **28**, 1697 (1945)
11 Billeter, J R , and Miescher, K *ibid* **29**, 859 (1946)
12 Birch, A J , Jaeger, R , and Robinson, R *J Chem Soc* **1945**, 582
13 Biskind G R *Endocrinology* **28**, 894 (1941), *Proc Soc Exptl Biol Med* **47**, 266 (1941)
14 Biskind, G R , and Mark, J *Bull Johns Hopkins Hosp* **65**, 212 (1939)
15 Biskind, G R , and Meyer, M A *Proc Soc Exptl Biol Med* **53**, 91 (1943)
16 Biskind, M S , and Biskind, G R *Endocrinology* **31**, 109 (1942)
17 Biskind M S , and Shelesnyak, M C *Endocrinology* **30**, 819 (1942)
18 Bloch, K , Berg B N , and Rittenberg, D *J Biol Chem* **149**, 511 (1943)
19 Browne, J S L , cited by Collip, J B *Proc Calif Acad Med* **1**, 38 (1931)
20 Burnop, V C E Elliot, G H , and Linstead R P *J Chem Soc* **1940**, 727
21 Butenandt, A *Naturwissenschaften* **17**, 878 (1929)
22 Butenandt, A *Z physiol Chem* **191**, 140 (1930)
23 Butenandt, A *Naturwissenschaften* **24**, 15 (1936)
24 Butenandt, A , Friedrich, W , and Poschmann L *Ber* **75**, 1931 (1942)
25 Butenandt, A , and Goergens, C *Z physiol Chem* **248**, 123 (1937)
26 Butenandt, A , and Hildebrandt, F *ibid* **199**, 243 (1931)
27 Butenandt, A , and Hofstetter, H *ibid* **259**, 222 (1939)
28 Butenandt, A , and Kudszus, H *ibid* **237**, 75 (1935)
29 Butenandt, A , and Poschmann L. *Ber* **77**, 394, (1944)
30 Butenandt, A , and Schramm G *ibid* **62**, 2083 (1935)
31 Butenandt, A , Wolff, H , and Karlson, P *ibid* **74**, 1308 (1941)
32 Cantarow, A , Paschkis, K E , Rakoff, A E , and Hansen L P *Endocrinology* **33**, 309 (1943)
33 Cantarow, A , Rakoff, A E , Paschkis, K E , and Hansen, L P *Proc Soc Exptl Biol Med* **49**, 707 (1942)
34 Cantarow, A , Rakoff, A E , Paschkis, K E , Hansen, L P , and Walkling, A A *ibid* **52**, 256 (1943)
35 Cantarow, A , Rakoff, A E , Paschkis K E , Hansen, L P , and Walkling, A A *Endocrinology* **31**, 515 (1942) **32**, 368 (1943)
36 Cohen, S L , and Marrian G F *J Soc Chem Ind* **54**, 1025 (1935), *Biochem J* **30**, 57 (1936)
37 *Cold Spring Harbor Symposia Quant Biol* V, 1937
38 Dane, E , and Eder, K *Ann* **539**, 207 (1939)
39 Dane, E , and Schmidt, J *ibid* **536**, 196 (1938), *ibid* **537**, 246 (1939)
40 David, K *Acta Brevia Néerland (Physiol Pharmacol Microbiol)* **4**, 63 (1934) *Chem Abstracts* **29**, 3384[1] (1935)
41 David, K *ibid.* **8**, 211 (1938)
42 De Jongh, S E , Kober, S , and Laqueur, E *Biochem Z* **240**, 247 (1931)
43 Deulofeu, V , and Ferrari, J *Z physiol Chem* **226**, 192 (1934)
44 Dingemanse, E , Laqueur, E , and Mühlbock, O *Nature* **141**, 927 (1938)
45 Dingemanse, E , and Tyslowitz, R *Endocrinology* **28**, 450 (1941)
46 Dirscherl, W *Z physiol Chem* **239**, 53 (1936)
47 Dirscherl W , and Hanusch, F *ibid* **236**, 139 (1935), *ibid* **233**, 13 (1935)
48 Doisy, E A , Thayer, S A , Levin, L , and Curtis, J M *Proc Soc Exptl Biol Med* **28**, 88 (1930)
49 Doisy, E A , Thayer, S A , and Van Bruggen, J T *Federation Proc* **1**, 202 (1942)

50 Doisy, E A, Veler, C D, and Thayer, S A *Am J Physiol* **90**, 329 (1929)
51 Drill, V A, and Pfeiffer, C A *Endocrinology* **38**, 300 (1946)
52 Emmens, C W *J Endocrinol* **2**, 444 (1941), **3**, 168, 174 (1942)
53 Engel, P, and Navratil, T *Biochem Z* **292**, 434 (1937)
54 Engel, P, and Rosenberg, E *Endocrinology* **37**, 44 (1945)
55 Fieser, L F Cause and Growth of Cancer, Univ of Pennsylvania Bicentennial Conference, U of Pennsylvania Press, Philadelphia, 1941, pp 1–27
56 Fieser, L F The Chemistry of Natural Products Related to Phenanthrene Reinhold, New York, 1936
57 Figge F H J *Endocrinology* **36**, 178 (1945)
58 Fish, W R, and Dorfman, R I *Science* **91**, 388 (1940), *J Biol Chem* **140**, 83 (1941)
59 Fish, W R, and Dorfman, R I *J Biol Chem* **143**, 15 (1942)
60 Freed, S, and Soskin, S *Endocrinology* **21**, 599 (1937)
61 Girard, A, Sandulesco, G,, Fridenson, A, Gaudefroy, C, and Rutgers, J J *Compt rend* **194**, 1020 (1932)
62 Girard, A, Sandulesco, G, Fridenson, A, and Rutgers, J J *ibid* **194**, 909 (1932), **195**, 981 (1932)
63 Glass, S J, Edmondson, H A, and Soll, S N *Endocrinology* **27**, 749 (1940)
64 Goldberg, M W, and Studer, S *Helv Chim Acta* **24**, 478 (1941)
65 Golden, J B, and Sevringhaus, E L *Proc Soc Exptl Biol Med* **39**, 361 (1938)
66 Graubard, M, and Pincus, G *Proc Natl Acad Sci U S* **27**, 149 (1941), *Federation Proc* **1**, 31 (1942), *Endocrinology* **30**, 265 (1942)
67 Gsell Busse, M A *Arch exptl Path Pharmacol* **139**, 328 (1929), *Klin Woch schr* **7**, 1606 (1928)
68 Gyorgy, P *Proc Soc Exptl Biol Med* **60**, 344 (1945)
69 Haussler, E P *Helv Chim Acta* **17**, 531 (1934)
70 Haussler, E P, Festschrift E C Barrell **1936**, 327 (Basel)
71 Heard, R D H, Bauld, W S, and Hoffman, M M *J Biol Chem* **141**, 709 (1941)
72 Heard R D H, and Hoffman, M M *ibid* **135**, 801 (1940), **138**, 651 (1941)
73 Heard R D H, and Hoffman, M M *ibid* **141**, 329 (1941)
74 Heer, J Billeter J R, and Miescher, K *Helv Chim Acta* **28**, 991 (1945), *ibid* **28**, 1342 (1945)
75 Heer, J, and Miescher, K *ibid* **28**, 156 (1945), **28**, 1506 (1945), **29**, 1895 (1946)
76 Heller, C G *Endocrinology* **26**, 619 (1940)
77 Heller, C G, and Heller, E J *ibid* **32**, 64 (1943)
78 Heller, C G Heller, E J, and Sevringhaus, E L *Am J Physiol* **126**, p 530 (1939)
79 Hirschmann, H *J Biol Chem* **150**, 363 (1943)
80 Hirschmann H, and Wintersteiner, O *ibid* **122**, 303 (1937–1938)
81 Hirschmann, H, and Wintersteiner, O *ibid* **126**, 747 (1938)
82 Honigmann H *Ann* **511**, 292 (1934)
83 Huffman, M N *J Am Chem Soc* **64**, 2235 (1942)
84 Huffman, M N and Darby, H H *ibid* **66**, 150 (1944)
85 Huffman, M N, and Lott, M H *J Biol Chem* **164**, 785 (1946)
86 Huffman, M N, and Miller, W R *Science* **100**, 312 (1944)
87 Huffman, M N, Thayer S A, and Doisy, E A *J Biol Chem* **133**, 567 (1940)
88 Hunter J H, and Hogg, J A *J Am Chem Soc* **68**, 1676 (1946)
89 Inhoffen H H *Ann* **497**, 130 (1932)

90 Inhoffen, H H, and Huang, M *Ber* **71**, 1720 (1938), **72**, 1686 (1939)
90a Inhoffen, H H, and Hohlweg, W *Naturwissenschaften* **26**, 96 (1938), Inhoffen, H H, Logemann, W, Hohlweg, W and Serini, A *Ber* **71**, 1024 (1938)
91 Inhoffen, H H, and Zühlsdorff, G *ibid* **74**, 1911 (1941)
92 Inhoffen, H H, Zühlsdorff, G, and Huang, M *ibid* **74**, 604 (1941)
93 Inhoffen, H H, Zühlsdorff, G, and Huang, M *ibid* **73**, 451 (1940)
94 Israel, S L, Meranze, D R, and Johnston, C G *Am J Med Sci* **194**, 835 (1937)
95 Johnson, W S, Petersen, J W, and Gutsche, C D *J Am Chem Soc* **67**, 2274, 1945
96 Jones E R H In *Ann Repts on Progress Chems (Chem Soc London)* XL, 122 (1943)
97 Koch F C *Endocrinology* **31**, 163 (1942)
98 Koebner, A, and Robinson, R *J Chem Soc* **1938**, 1944 **1941**, 566
99 Koref, O, and Engel, P *Endocrinology* **38**, 133 (1946)
100 Levin, L *J Biol Chem* **157**, 407 (1945)
101 Levin, L *ibid* **158**, 725 (1945)
102 Litvan, F, and Robinson, R *J Chem Soc* **1938**, 1997
103 Longwell, B B, and McKee, F S *J Biol Chem* **142**, 757 (1942)
104 Longwell B B, and Wintersteiner, O *ibid* **133**, 219 (1940)
105 MacCorquodale, D W, Levin, L, and Thayer, S A *ibid* **105**, LV (1934)
106 MacCorquodale, D W, Levin, L, Thayer, S A, and Doisy, E A *ibid* **101**, 753 (1933)
107 MacCorquodale, D W, Thayer, S A, and Doisy, E A *ibid* **99**, 327 (1932–1933)
108 MacCorquodale, D W, Thayer, S A, and Doisy, E A *ibid* **115**, 435 (1936)
109 Marker, R E *J Am Chem Soc* **60**, 1897 (1938)
110 Marker, R. E *ibid* **61**, 944, 1287 (1939)
111 Marker, R E, and Hartman, C G *J Biol Chem* **133**, 529 (1940)
112 Marker, R E., Kamm, O, Oakwood, T S, and Laucius, J F *J Am Chem Soc* **58**, 1503 (1936)
113 Marker, R E, Kamm, O, Oakwood, T S, and Tendick, F H *ibid* **59**, 768 (1937)
114 Marker, R E, and Rohrmann, E *ibid* **61**, 2537 (1939)
115a Marker, R E, and Rohrmann, E *ibid* **60**, 2927 (1938)
115b Marker, R E, and Rohrmann, E *ibid* **61**, 3314 (1939)
115c Marker, R L, and Rohrmann, E *ibid* **62**, 73 (1940)
116 Marker, R T Rohrmann, E, Lawson, E J, and Wittle, E L *ibid* **60**, 1901 (1938)
117 Marker, R E, Rohrmann, E, Wittle, E L, and Lawson, E J. *ibid* **60**, 1512 (1938)
118 Marker, R E, Rohrmann, E, Wittle, E L, and Tendick, F H *ibid* **60**, 2440 (1938)
119 Marrian, G F *J Soc Chem Ind* **49**, 515 (1930)
120 Marrian, G F *Endeavour* **5**, 35 (1946)
121 Marrian, G F, and Haslewood, G A D *J Soc Chem Ind* **51**, 277 T (1932).
122 Marrian, G F, and Haslewood, G A D *Biochem J* **26**, 25 (1932)
123 Miescher, K *Helv Chim Acta* **27**, 1727 (1944)
124 Moss A R, and Schoenheimer, R *J Biol Chem* **135**, 415 (1940)
125 Mühlbock, O *Z physiol Chem* **250**, 139 (1937)
126 Mühlbock, O *Lancet* I, 634 (1939)

127 Office of the Publication Board, Department of Commerce, Washington, D C, Report No 245
128 Office of the Publication Board, Department of Commerce, Washington, D C Report No 248, Pharmaceuticals at the I G Farbenindustrie Plant, Elberfield, Germany
129 Paschkis, K E, Cantarow, A, Rakoff, A E, Hansen, L P, and Walkling, A A *Proc Soc Exptl Biol Med* **53**, 213 (1943)
130 Paschkis, K E, Cantarow, A, Rakoff, A E, Hansen, L P, and Walkling A A *ibid* **55**, 127 (1944)
131 Pearlman, W H, Paschkis, K E, Rakoff, A E, Cantarow, A, Walkling A A and Hansen, L P *Endocrinology* **36**, 284 (1945)
132 Pearlman, W H, and Pearlman, M R J *Arch Biochem* **4**, 97 (1944)
133 Pearlman, W H, and Pincus, G *J Biol Chem* **144**, 569 (1942)
134 Pearlman, W H, and Pincus, G *ibid* **147**, 379 (1943)
135 Pearlman, W H, and Wintersteiner, O *ibid* **130**, 35 (1939)
136 Pearlman, W H, and Wintersteiner, O *ibid* **132**, 605 (1940)
137 Pincus, G, and Martin, D W *Endocrinology* **27**, 838 (1940)
138 Pincus, G, and Pearlman, W H *ibid* **31**, 507 (1942)
139 Pincus, G, and Pearlman, W H *Vitamins and Hormones* **1**, 294 (1943)
140 Pincus G, and Zahl, P A *J Gen Physiol* **20**, 879 (1937)
141 Prelog V, and Fuhrer, J *Helv Chim Acta* **28**, 583 (1945)
142 Prelog, V, Ruzicka, L, and Wieland, P *ibid* **28**, 250 (1945)
143 Rakoff, A E, Paschkis, K E, and Cantarow, A *Am J Obstet Gynecol* **46**, 856 (1943)
144 Raper, H S *Physiol Revs* **8**, 245 (1928)
145 Remezof, I *Rec trav chim* **55**, 797 (1936)
146 Roberts, S, and Szego, C M *Endocrinology* **39**, 183 (1946)
147 Roberts, S, and Szego, C M (In press)
148 Robinson, R, and Rydon, H N *J Chem Soc* **1939**, 1395
149 Rometsch R, and Miescher, K *Helv Chim Acta* **29**, 1231 (1946)
150 Rondoni P, Carminati, V, and Corbellini, A *Z physiol Chem* **241**, 71 (1936)
151 Ruzicka, L, Müller, P, and Morgeli, E *Helv Chim Acta* **21**, 1394 (1938)
152 Ruzicka L, Prelog V, and Wieland, P *ibid* **28**, 1609 (1945)
153 Samuels, L T, and McCauley, C J *Endocrinology* **39**, 78 (1946)
154 Schacter, B and Marrian, G F *J Biol Chem* **126**, 663 (1938)
155 Schiller, J, and Pincus, G *Science* **98**, 410 (1943), *Arch Biochem* **2**, 317 (1943)
156 Schiller J, and Pincus, G *Endocrinology* **34**, 203 (1944)
157 Schoeller, W, Schwenk, E, and Hildebrandt, F *Naturwissenschaften* **21**, 286 (1933)
158 Schwenk E *U S Pat* 2 294, 938, Sept 8, 1942
159 Schwenk E, and Hildebrandt, F *Naturwissenschaften* **21**, 177 (1933)
160 Segaloff A *Endocrinology* **33**, 209 (1943), **34**, 335 (1944)
161 Segaloff A *ibid* **38**, 212 (1946)
162 Segaloff, A and Nelson, W O *Proc Soc Exptl Biol Med* **48**, 33 (1941) *Federation Proc* **1**, 79 (1942)
163 Segaloff, A, and Segaloff, Ann *Endocrinology* **34**, 346 (1944)
164 Selye, H *ibid* **30**, 437 (1942)
165 Serini, A and Logemann W *Ber* **71**, 186 (1938)
166 Shipley R A and Gyorgy, P *Proc Soc Exptl Biol Med* **57**, 52 (1944)
167 Singher H O, Kensler, C J, Taylor, H C, Jr, Rhoads, C P, and Unna, K *J Biol Chem.* **154**, 79 (1944)

168 Smith, G V S, and Smith, O W *Am J Obstet Gynecol* **36**, 769 (1938)
169 Smith, G V S, Smith, O W, Huffman, M N, MacCorquodale, D W, Thayer, S A, and Doisy, E A *J Biol Chem* **130**, 431 (1939)
170 Smith, O W *Endocrinology* **35**, 146 (1944)
171 Smith, O W *Proc Soc Exptl Biol Med* **59**, 212 (1945)
172 Smith, O W, and Smith, G V S *Endocrinology* **28**, 740 (1941)
173 Solmssen, U V *Chem Revs* **37**, 481 (1945)
174 Spoor, H J, and Ralli, E P *Endocrinology* **35**, 325 (1944)
175 Stroud, S W *J Endocrinol* **1**, 201 (1939)
176 Stroud, S W *ibid* **2**, 55 (1940)
177 Szego, C M, and Barnes, R H *Endocrinology* **32**, 367 (1943)
178 Szego, C M, and Roberts, S *ibid* **36**, 104 (1945)
179 Szego, C M, and Roberts S *Proc Soc Exptl Biol Med* **61**, 161 (1946)
180 Szego, C M, and Samuels, L T *J Biol Chem* **151**, 599 (1943)
181 Talbot, N B *Endocrinology* **25**, 601 (1939)
182 Thayer, S A, MacCorquodale, D W, and Doisy, E A *J Pharmacol* **59**, 48 (1937)
183 Twombly, G H, and Taylor, H C, Jr *Cancer Research* **2**, 811 (1942)
184 *Vitamins and Hormones* **4**, 135–146 and 147–185 (1946)
185 Wakim, K G, and Mann, F C *Arch Path* **33**, 198 (1942)
186 Weidlich, H A, and Meyer-Delius, M *Ber* **74**, 1195, 1213 (1941)
187 Westerfeld, W W *Biochem J* **34**, 51 (1940)
188 Westerfeld, W W *J Biol Chem* **143**, 177 (1942)
189 Westerfeld, W W, and Doisy, E A *Ann Internal Med* **11**, 267 (1937)
190 Westerfeld, W W, MacCorquodale, D W, Thayer, S A, and Doisy, E A *J Biol Chem* **126**, 195 (1938)
191 Westerfeld, W W, Thayer, S A, MacCorquodale, D W, and Doisy, E A *ibid* **126**, 181 (1938)
192 Whitman, B, Wintersteiner, O and Schwenk, E *ibid* **118**, 789 (1937)
193 Wilds, A L, Beck, L W, and Johnson, T L *J Am Chem Soc* **68**, 2161 (1946)
194 Wilds, A L, and Djerassi, C *ibid* **68**, 2125 (1946)
195 Windaus, A, and Borgeaud, P *Ann* **460**, 235 (1928)
196 Windaus, A, and Deppe, M *Ber* **70**, 76 (1937)
197 Wintersteiner, O *J Am Chem Soc* **59**, 765 (1937)
198 Wintersteiner, O, Schwenk, E, Hirschmann, H, and Whitman, B *ibid* **58**, 2652 (1936)
199 Wintersteiner, O, Schwenk, E, and Whitman, B *Proc Soc Exptl Biol Med* **32**, 1087 (1935)
200 Zondek, B *Arkiv Kemi Mineral Geol*, **11B**, No 24 (1933), *Nature* **133**, 209, 494 (1934)
201 Zondek, B Hormone des Ovariums und des Hypophysenvorderlappens 2nd ed Springer, Vienna 1935 p 124 *Skand Arch Physiol* **70**, 133 (1934)
202 Zondek, B Clinical and Experimental Investigations on the Genital Functions and their Hormonal Regulation Williams and Wilkins, Baltimore, 1941, pp 145–157
203 Zondek, B, and Sklow, J *Proc Soc Exptl Biol Med* **46**, 276 (1941)
204 Zondek, B, and Sulman, F *Endocrinology* **33**, 204 (1943)
205 Zondek, B, and Sulman, F *Vitamins and Hormones* **3**, 317 (1945)
206 Zondek, B, Sulman, F, and Sklow, J *Endocrinology* **33**, 333 (1943), *Nature* **143**, 282 (1939)

Chapter XI

Chemistry and Metabolism of Progesterone

By WILLIAM H PEARLMAN

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I. Chemistry of Progesterone

A Partial Synthesis of Progesterone

Many steroids occurring in nature may serve as starting material for the preparation of progesterone (V) Those steroids containing more than 21 carbon atoms obviously require the degradation of the C_{17} side chain in order to obtain derivatives of pregnane (C_{21} series), the latter compounds may then be converted to progesterone In the process of shortening the side chain, C_{19} steroids (and other steroids) may be formed, these may be utilized for the preparation of progesterone or for the preparation of other steroid hormones, *e g*, testosterone (CXXI), desoxycorticosterone (LXL), etc

1 *From Cholesterol*

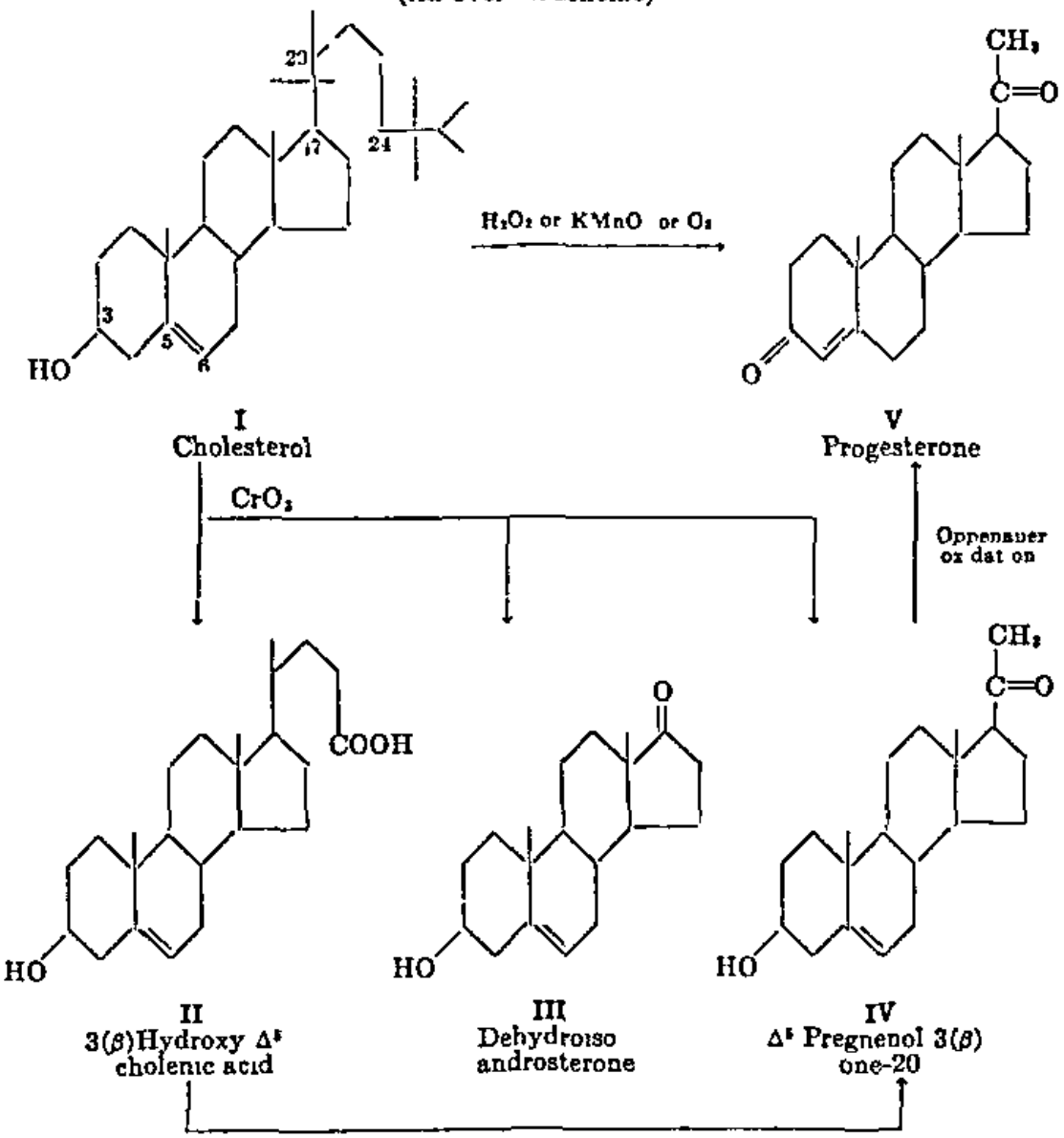
Drastic oxidation of cholesterol (I) with chromic acid results in the formation, in low yield, of dehydroisoandrosterone (III), 3(β)-hydroxy-Δ^5 cholenic acid (II) (Butenandt *et al*, 19, Wallis and Fernholz, 221), Δ^5-pregnenol-3(β)-one-20 (IV) (Fujii and Matsukawa, 64, Ruzicka and Fischer, 185, Schwenk and Whitman, 198) as well as other products The following groups in cholesterol are shielded during the process of oxidation (*a*) the hydroxyl group on carbon 3 by acetylation, (*b*) the ethylenic double bond by preparation of the 5,6-dibromide, debromination is subsequently effected with zinc dust and acetic acid The oxidation products (see Chart 1) are converted to progesterone by procedures described below

Progesterone may also be obtained from cholesterol in one major operation but the yields are very poor For example, Serono and Marchetti (201) treated the 5,6-dibromo derivative of cholesterol (10 g) with hydrogen peroxide in an alkaline solution containing a trace of moist silver oxide, 80 mg of a semicrystalline mass was obtained which possessed progestational activity Spielman and Meyer (206) treated a benzene solution of cholesterol dibromide with aqueous acid permanganate, the yield of progesterone was about 0 2% on the basis of bioassay Although the yield appears to be infinitesimal, the authors claim that the cost is a fraction of that required by the then current methods for obtaining progesterone The preparation of crystalline progesterone is not feasible, however, by this procedure because of the inherent difficulties in purification Cholestenone is obtained in about 50% yield in the process just described, it can be converted to progesterone by oxidation with chromic acid by a procedure described by Dirscherl and Hanusch (44) Tavastsherna (212) oxidized cholestenone dibromide (obtained from 10 g cholesterol) in benzene solution by shaking with an aqueous acid perman-

ganate solution, 40–60 mg progesterone were finally obtained, Spielman and Meyer (206) were unable to duplicate these findings Bretschneider (13) passed oxygen through molten cholestenone at 170° in the presence of vanadium pentoxide, an unspecified small amount of progesterone was obtained

CHART 1

THE PARTIAL SYNTHESIS OF PROGESTERONE (V) FROM CHOLESTEROL (I)
(An over all scheme)



2 *From Stigmasterol*

Fernholz (59) treated the 5,6-dibromo derivative of stigmasterol acetate with ozone, thereby rupturing the double bond between carbon atoms 22 and 23, debromination and hydrolysis yielded the corresponding

bisnorcholenic acid (XIII) The latter compound was subsequently converted to progesterone (see Section I, A, 4)

Butenandt *et al* (19) oxidized stigmasterol and obtained dehydroisoandrosterone (III) by a procedure essentially that followed in obtaining III from cholesterol (I), other plant sterols of like nature, *e g*, sitosterol, may be similarly degraded The 17-ketosteroid thus obtained may be utilized for preparation of progesterone (see Section I, A, 6)

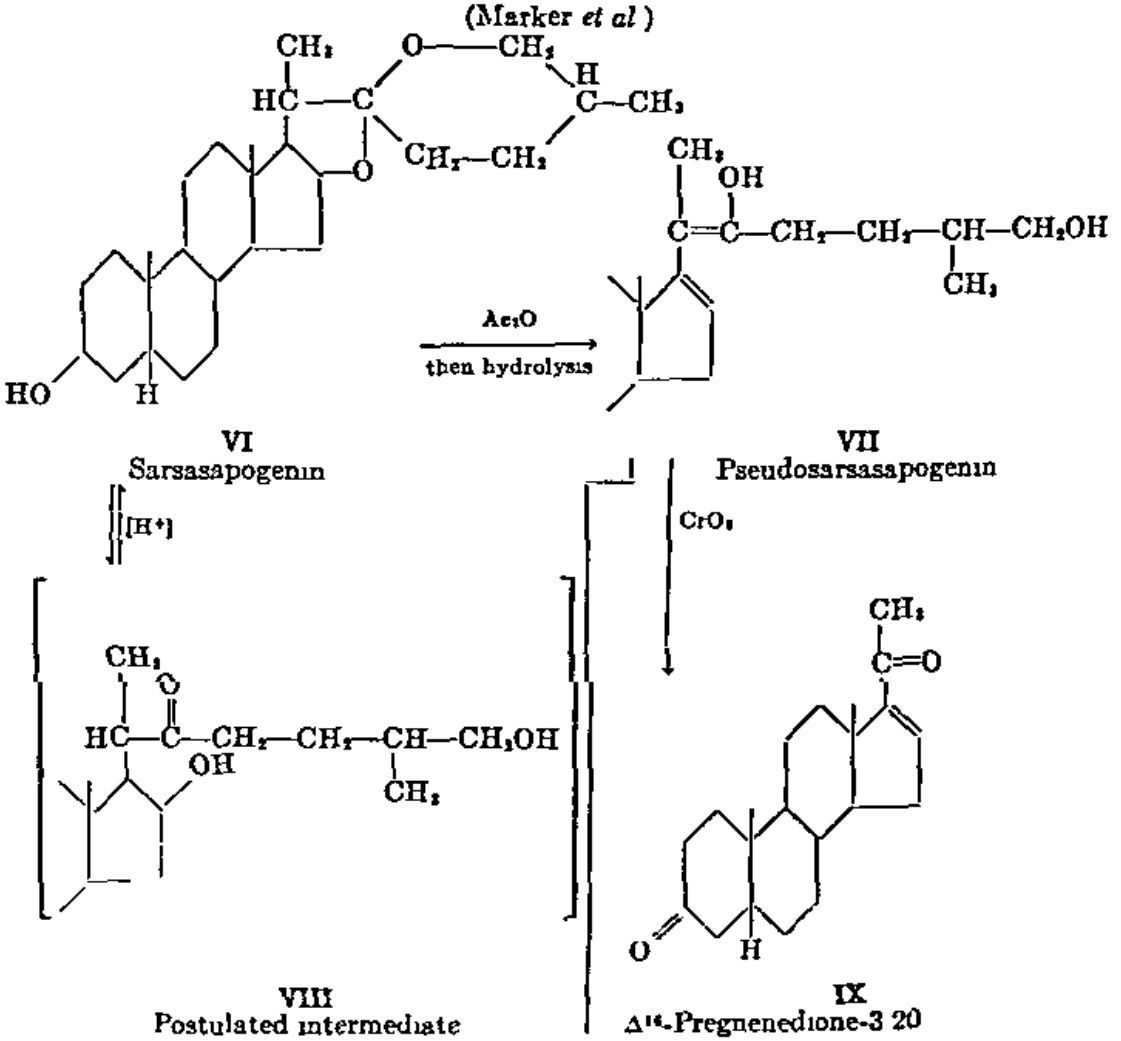
3 *From Sapogenins*

Marker and co-workers (110,111,136–139,143) utilized sapogenins for the preparation of pregnane compounds, the yields are high Thus, Δ^{16}-pregnenol-3(α)-one-20 is obtained in 52% yield from episarsasapogenin acetate (Marker, 111) In view of the fact that this compound and certain other C_{21} steroids may be converted to progesterone (V)

CHART 2

DEGRADATION OF SAPOGENINS TO PREGNANE DERIVATIVES

(Marker *et al*)

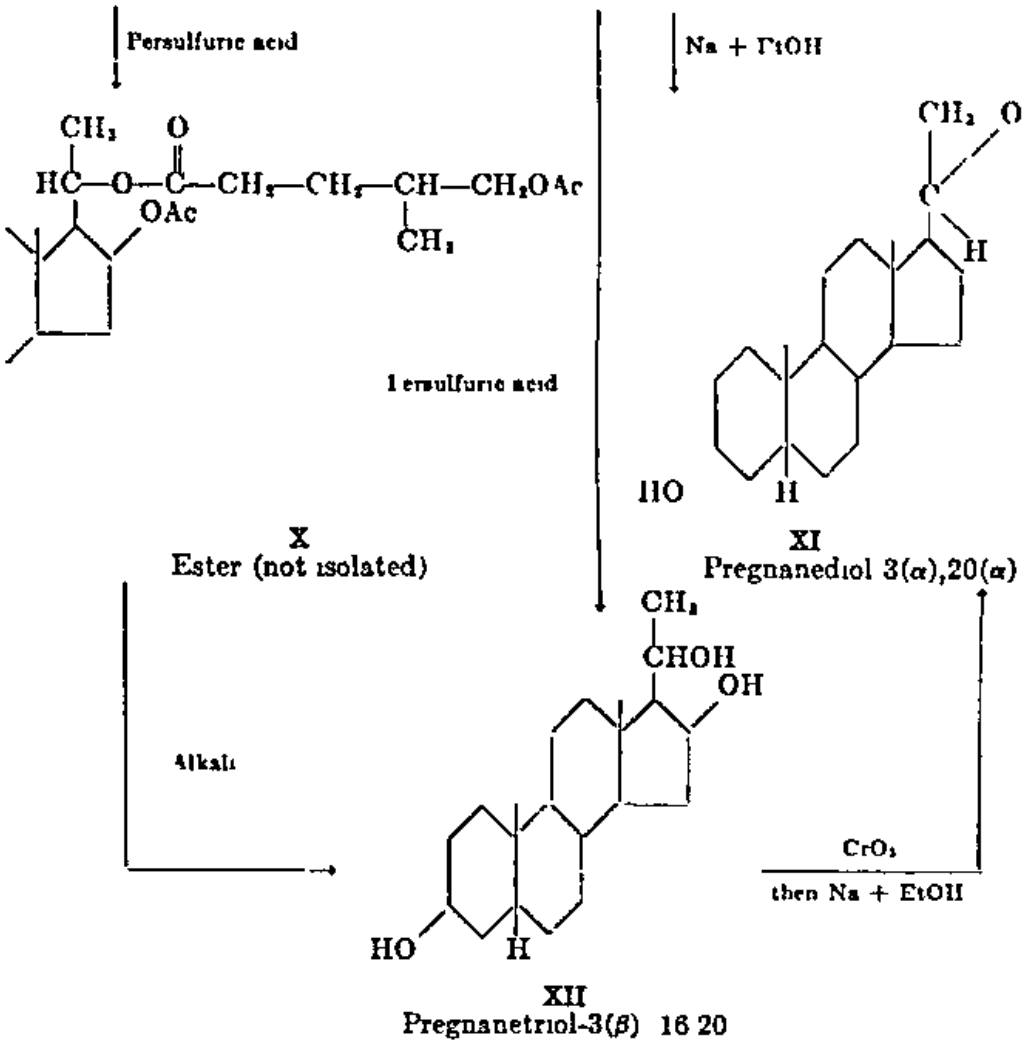


VI
Sarsasapogenin

VII
Pseudosarsasapogenin

VIII
Postulated intermediate

IX
Δ^{16}-Pregnenedione-3 20



without great difficulty (see Section I, A, 5), the sapogenins appear to be a very suitable source for the preparation of progesterone As an illustration of the degradative procedures employed by Marker and co-workers, the conversion of sarsasapogenin (VI) to Δ^{16}-pregnenedione-3,20 (IX) is now described (see Chart 2) The starting product, VI, is heated with acetic anhydride and the reaction product hydrolyzed to obtain pseudosarsasapogenin (VII) Mild oxidation of VII with chromic acid gives Δ^{16}-pregnenedione-3,20 (IX) (Marker and Rohrmann, 136) In a similar manner, Δ^{16}-allopregnenedione-3,20 (CXI) may be obtained from tigogenin (Marker and Rohrmann, 137), and $\Delta^{5\,16}$-pregnadienol-3(β)-one-20 (XLIV) from diosgenin (Marker *et al*, 143)

The sapogenins may be degraded to C_{21} steroids by yet another method Thus, for example, sarsasapogenin acetate (VI acetate) on treatment with persulfuric acid followed by hydrolysis of the reaction product (not isolated) yields pregnanetriol-3(β),16,20 (XII) in 20–40% yields XII, on chromic acid oxidation followed by reduction with

sodium and alcohol, yields pregnanediol-3(α),20(α) (XI) (Marker *et al*, 138) (see Chart 2)

4 *From Bile Acids*[1]

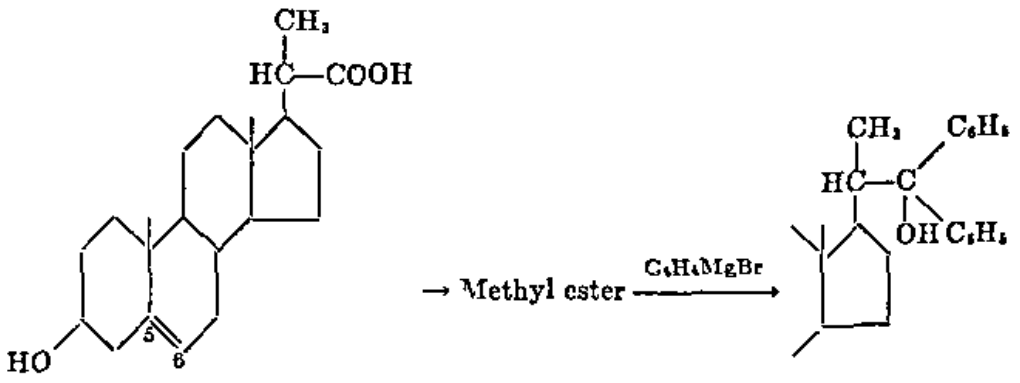
Fernholz (60) treated the methyl ester of 3(β)-hydroxy-Δ^5-bisnorcholenic acid (XIII) (derived from stigmasterol) with phenyl magnesium bromide to obtain the diphenylcarbinol, XIV Dehydration of XIV yields XV, the Δ^5 double bond of XV is protected by selective bromination and the Δ^{20} double bond is then ruptured by ozonolysis Dehalogenation of the oxidation product yields Δ^5 pregnenol-3(β)-one-20 (IV) from which progesterone (V) is readily derived Butenandt *et al* (36), independently of Fernholz, prepared progesterone by the same route Erhart *et al* (57) also succeeded in converting XIII to progesterone, XIII was degraded, in this instance, by the method of Curtius

The naturally occurring bile acids serve as an excellent source for the preparation of pregnane derivatives Efforts aimed at shortening the side chain in bile acids have been greatly intensified in recent years with the main objective of preparing etiocholanic acids for the partial synthesis of adrenal cortical hormones (for a fuller discussion, see Chapter XIII) As intermediates in the degradative process, pregnane derivatives may be obtained Thus, for example, Hoehn and Mason (85), by introducing certain modifications in the Barbier Wieland method (8), greatly increased the yield of 20 ketopregnane compounds derived from bile acids The steps involved (see Chart 3), which may be compared with

CHART 3

CONVERSION OF BILE ACIDS TO PROGESTERONE (V) (AND OTHER 20 KETO PREGNANE COMPOUNDS)

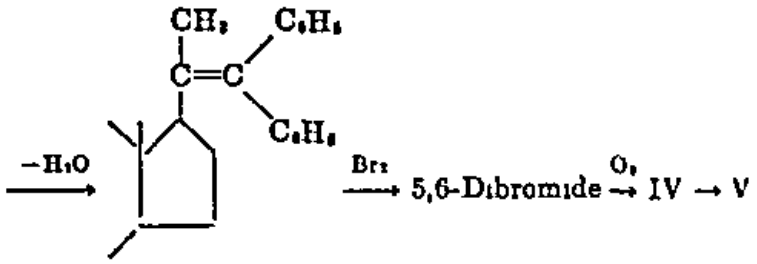
A Fernholz Butenandt *et al*



XIII
3(β)Hydroxy Δ^5 bisnorcholenic acid (derived from stigmasterol)

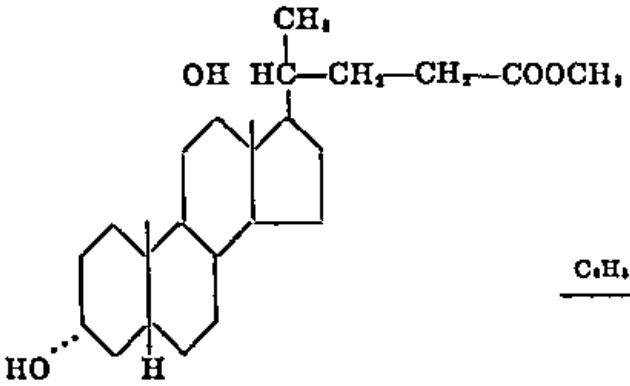
XIV
"Diphenyl carbinol"

[1] See Chart 3

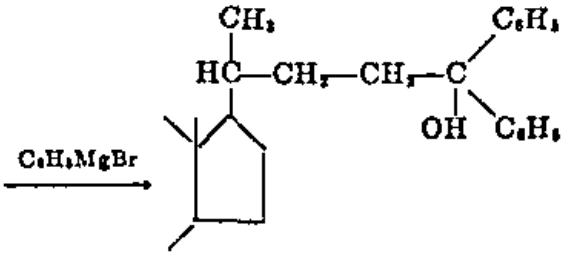


XV

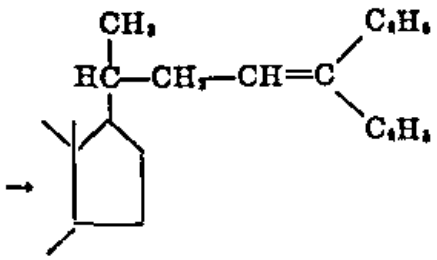
B Hoehn and Mason



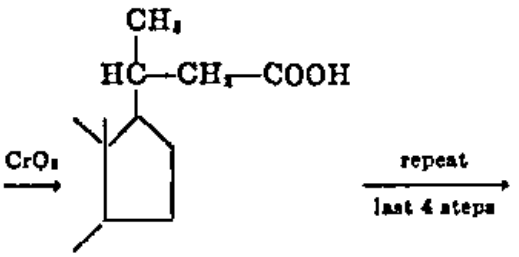
XVI
Desoxycholic acid, methyl ester



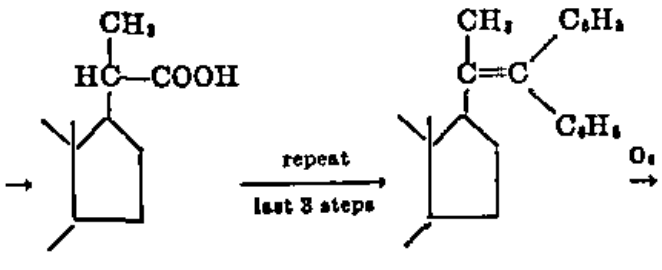
XVII
"Diphenylcarbinol"



XVIII

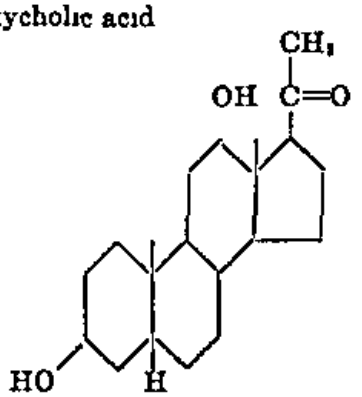


XIX
Nordesoxycholic acid



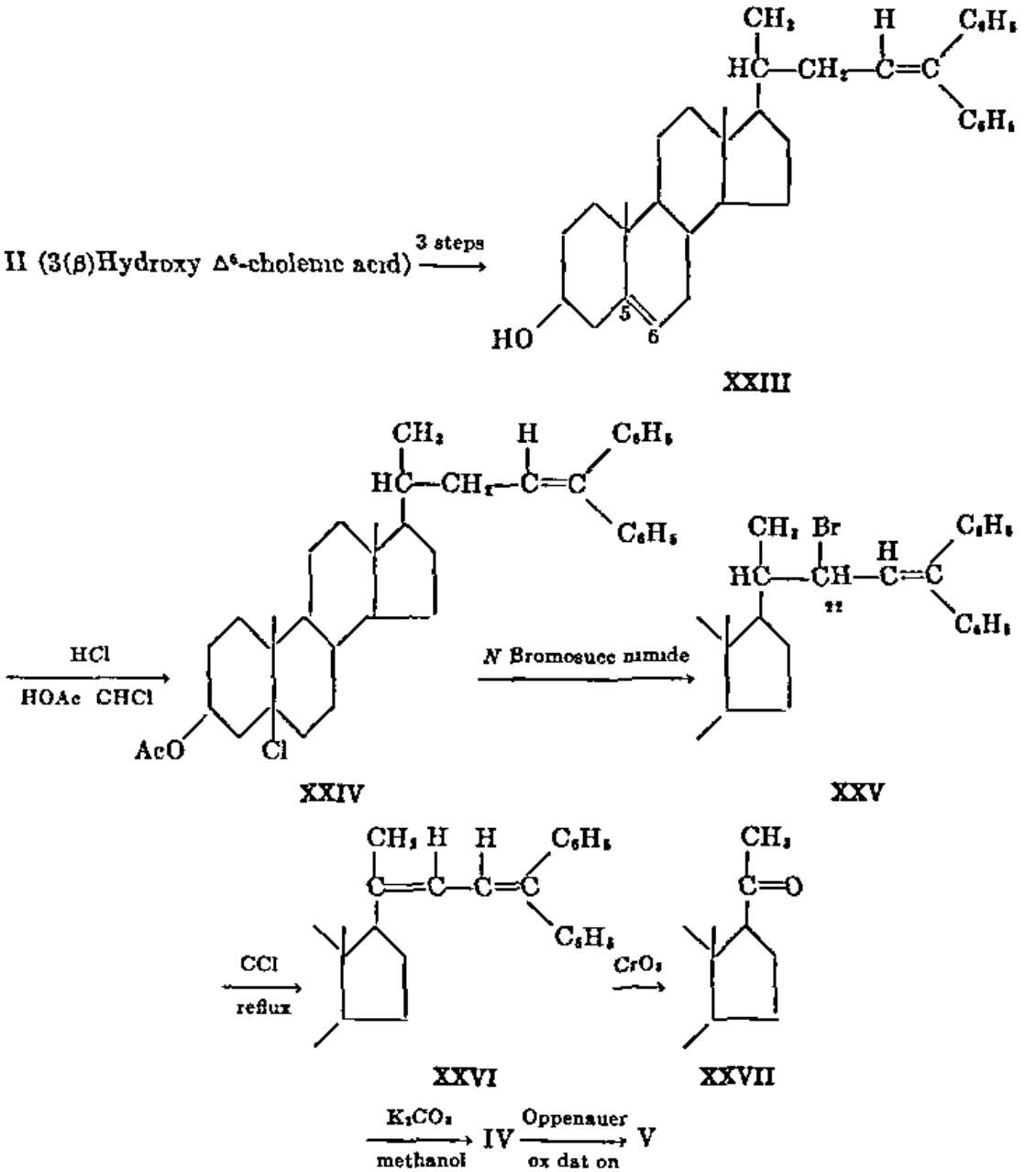
XX
Bisnordesoxycholic acid

XXI



XXII
Pregnanediol-3(α), 12(α)-one-20

C Meystre Miescher *et al*



those in Fernholz s procedure for converting 3(β) hydroxy Δ⁵ bisnor cholenic acid (II) to Δ⁵ pregnenol-3(β)-one-20 (IV), are as follows the methyl ester of desoxycholic acid (XVI) is treated with phenyl magnesium bromide to obtain the corresponding diphenyl carbinol XVII XVII readily undergoes dehydration which results in the formation of a double bond between carbon atoms 23 and 24 (XVIII), chromic acid oxidation ruptures this double bond and nordesoxycholic acid (XIX) is formed Repetition of the foregoing process yields the bisnor acid XX The

process is once more repeated but instead of chromic acid, ozone is employed to rupture the double bond which is now between carbon atoms 20 and 22 (in XXI) The product finally obtained is pregnanediol-3(α),12(α)-one-20[3] (XXII) If lithocholic acid is substituted for desoxycholic acid in the foregoing scheme, pregnanol-3(α)-one-20 (LVIII) is the final product Hoehn and Mason (46) were also successful in increasing the yield in the preparation of lithocholic acid from cholic acid (or from desoxycholic acid) Thus, the path to progesterone via cholic acid, a most readily accessible bile acid, is considerably improved Marker and Krueger (68) succeeded in converting hyodesoxycholic acid to progesterone The side chain in this bile acid was shortened by the method of Barbier-Wieland as described by Hoehn and Mason (85), the pregnanediol-3(α),6(β)-one-20[4] thus obtained was oxidized to pregnanol-6(β)-dione-3,20 The 6-acetyl derivative of the latter substance underwent dehydration on distillation over potassium hydrogen sulfate, yielding progesterone V The yield in the conversion of pregnanediol-3(α),6(β)-one to progesterone might be considerably improved if the novel procedure described by Gallagher and Xenos (66) were applied The latter investigators converted hyodesoxycholic acid to the corresponding α,β-unsaturated ketone by submitting the methyl ester of the bile acid to gentle Oppenauer oxidation This resulted in the preferential oxidation of the 3-hydroxyl group to give methyl-3-keto-6-hydroxycholenate The 6-*p*-tosyl-derivative of the latter compound on heating with collidine yielded methyl-3-keto-Δ^4-cholenate Kimura and Sugiyama (95) also prepared pregnanediol-3(α),6(β)-one-20[4] from hyodesoxycholic acid The side chain in the bile acid was shortened by the method of Wieland, Schlichtling, and Jacobi (8), *i e*, the dimethylcarbinols were prepared rather than the diphenylcarbinols and these were not dehydrated prior to chromic acid oxidation (see methods described above)

The procedures recently devised by Meystre, Miescher, and co-workers (158–162) for converting bile acids to the corresponding 20-ketopregnane derivatives appear to be most expedient in view of the high yields reported As an example, the conversion of 3(β)-hydroxy-Δ^5-cholenic *acid (II), a by-product in the oxidation of cholesterol (I), to* Δ^5*-pregnenol-*3(β) one-20 (IV), will be briefly described (Meystre *et al*, 159) (see Chart 3C) II is converted to XXIII (see procedure for conversion of XVI to XVIII in Chart 3B) Treatment of XXIII with hydrochloric

[3] The hydroxyl group on carbon 12 is designated α rather than β in view of the recent evidence that in desoxycholic acid this group is α (Gallagher and Long Sorkin and Reichstein, 65)

[4] Pregnanediol-3(α),6(β)-one 20 was recently prepared by Moffett *et al* (165) from hyodesoxycholic acid by two independent methods, but it did not appear to be identical with the products obtained by either of the investigators cited above

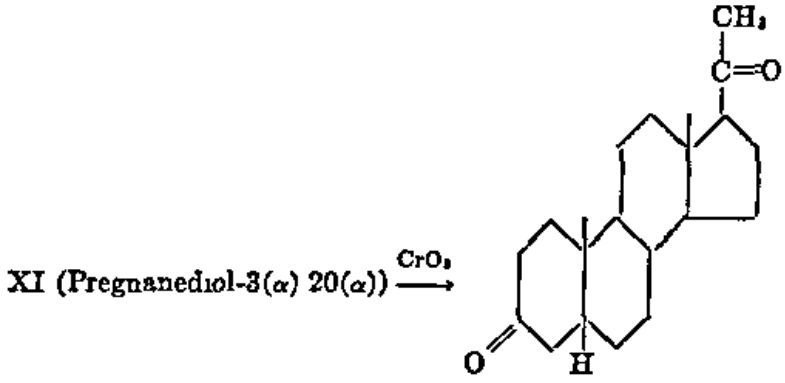
acid results in the selective addition of the reagent to the 5,6 double bond, giving the 5 chloro derivative, XXIV, the over-all yield (II to XXIV) is 79% The last step (XXIII to XXIV) is carried out to protect the 5,6 double bond in the following manipulations XXIV is treated with *N*-bromosuccinimide, thereby introducing a bromine atom on carbon 22 (in XXV) The bromo derivative, XXV, is refluxed with carbon tetrachloride whereupon hydrobromic acid is eliminated, giving XXVI, XXVI, on oxidation with chromic acid, gives the corresponding 20 keto steroid, XXVII, which, on treatment with potassium carbonate in methanol, forms Δ^5 pregnenol-3(β) one 20 (IV) Conversion of the latter to progesterone (V) is easily accomplished by Oppenauer oxidation, the over-all yield of V from XXIV is 43% Pregnanol-3(α)-one-20 (LVIII) may be obtained by a similar degradation of the side chain in lithocholic acid (Meystre and Miescher, 162), other bile acids have been subjected to similar treatment (Meystre *et al*, 158,160,161, Moffett *et al*, 165)

Reich and Reichstein (181) treated the diacetate of desoxycholic acid

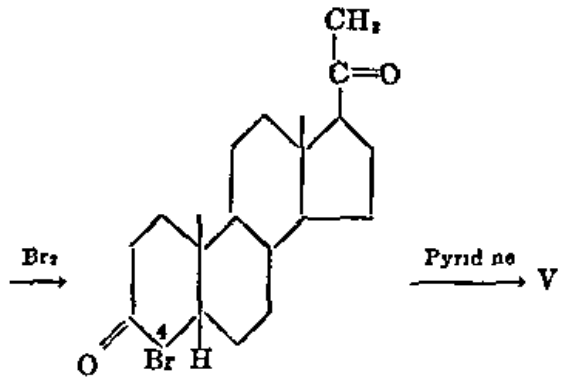
CHART 4

CONVERSION OF (ALLO) PREGNANE COMPOUNDS TO PROGESTERONE (V)

A Pregnane Series

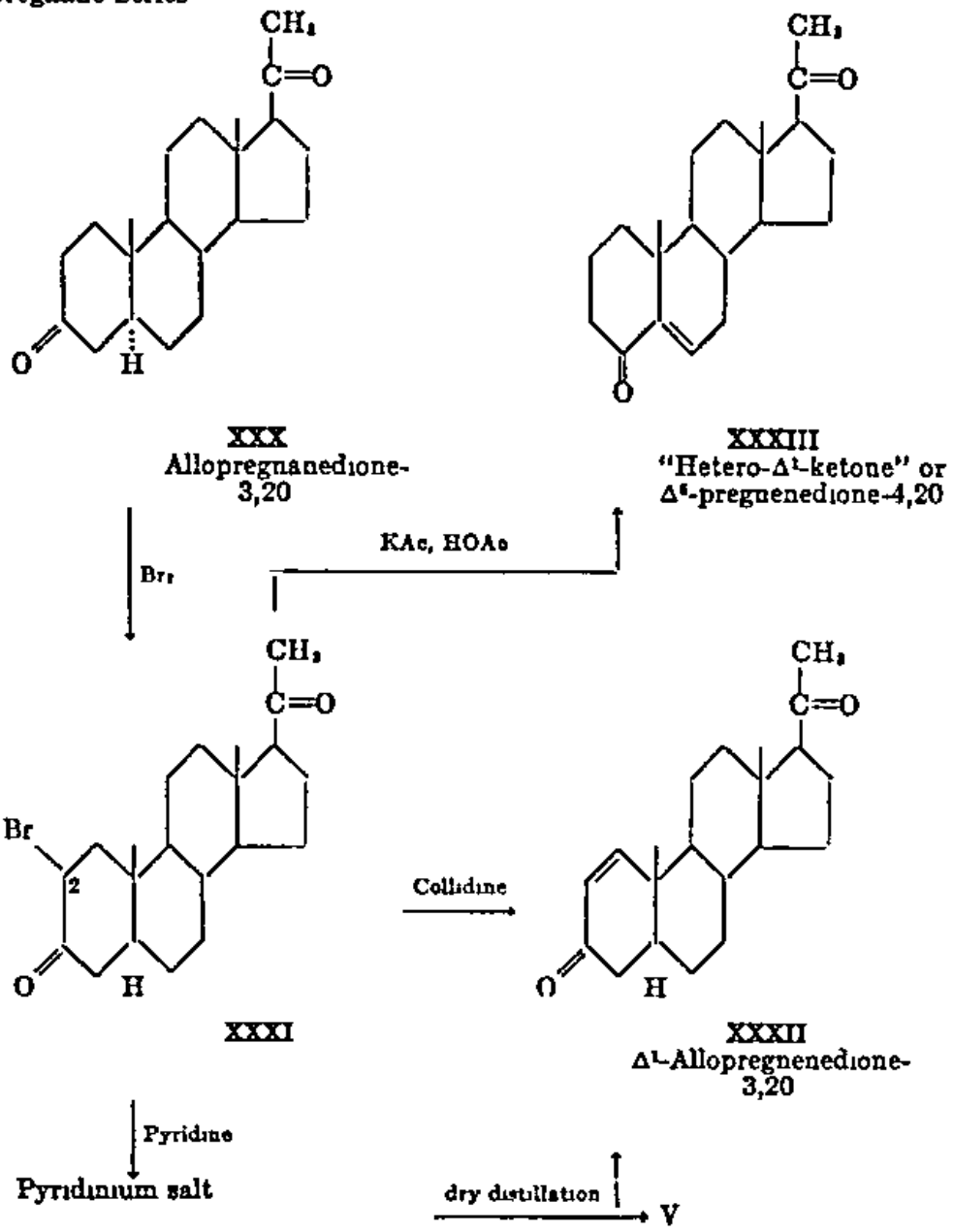


XXVIII
Pregnanedione 3 20



XXIX
4 Bromo derivative

B. Allopregnane Series



(XVI) with chromic acid; a very small amount of the corresponding 20-ketopregnane derivative, XXII, was obtained. Apparently, direct oxidative attack on compounds of the cholanic acid series is of little preparative service in this connection.

5. *From Compounds in the Pregnane and Allopregnane Series*

Substances belonging to the pregnane and allopregnane series may be utilized for the partial synthesis of progesterone. These compounds

may be obtained from urinary extracts (see Section II), and by degradation of the sapogenins (see Section I, A, 3) or of the bile acids (see Section I, A, 4)

Butenandt and Schmidt (31) succeeded in converting pregnanediol 3(α),20(α) (XI) to progesterone (V) (see Chart 4) The partial synthesis was accomplished by oxidation of XI to the corresponding dione, XXVIII, bromination at position 4 (XXIX) and treatment of XXIX with collidine or pyridine to eliminate hydrogen bromide

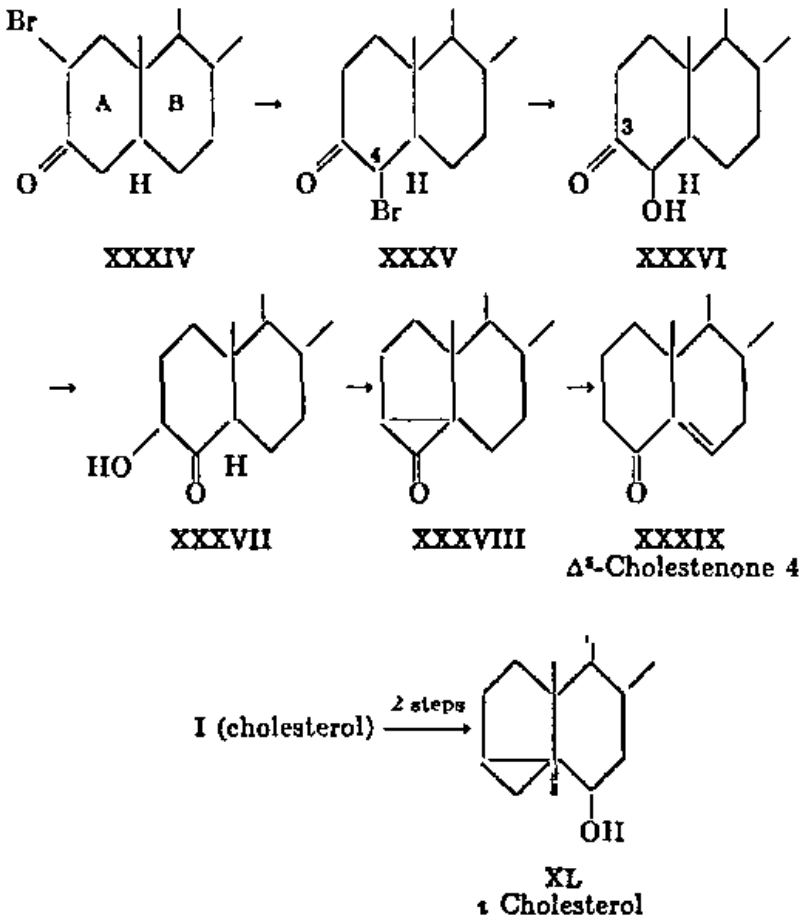
In contrast to pregnanedione-3,20 (XXVIII) (rings A and B *cis*), allopregnanedione-3,20 (XXX) (rings A and B *trans*) yields the 2 bromo derivative, XXXI, and not a 4-bromo derivative (Butenandt and Mamoli, 23), bromination of analogous compounds in the bile acid and cholesterol series follows a similar course (Butenandt *et al*, 25) XXXI resists dehalogenation with pyridine, forming a stable pyridinium salt which, on dry distillation, yields a mixture of approximately equal quantities of progesterone (V) and its Δ^1 isomer (XXXII) (Marker *et al*, 150) XXXII may also be obtained by treating 2-bromoallopregnanedione-3,20 (XXXI) with collidine (Butenandt *et al*, 26) But if XXXI is heated with potassium acetate in acetic acid, an α,β unsaturated ketone, XXXIII, is obtained which is not identical with any of the above mentioned substances XXXIII was tentatively labeled by Butenandt *et al* (26) as the hetero-Δ^1 ketone (or H Δ^1-ketone), it had been previously erroneously designated as Δ^1-pregnenedione-3,20 The structure of the H Δ^1-ketone has recently been elucidated by Butenandt and Ruhenstroth-Bauer (29), as model substance, H-Δ^1 cholestenone (XXXIX) was prepared from 2 bromocholestanone-3 (XXXIV) Hydrogenation of an alcoholic solution of XXXIX, in the presence of Raney nickel and orthoformic ester, yielded cholestanone-4 [4] This observation established the position of the carbonyl group at carbon 4 in the H-Δ^1-ketone The position of the double bond may be deduced from ultraviolet absorption data in the following manner the peak of absorption of the heterosteroid is at 240 mμ (as is the case with progesterone) and therefore the double bond must be in a position α,β with respect to the carbonyl group Of the two possible positions for this double bond the Δ^5 position is more probable since steroids of type XXXII absorb at 230 mμ Indeed, Clemmensen reduction of the H Δ^1-ketone gives Δ^5-cholestene, and treatment of the H-Δ^1 ketone with potassium permanganate in acetone solution yields cholestanediol 5,6-one-4 The structure of H Δ^1-cholestenone

[4] Hydrogenation of the H Δ^1 ketone in acetic acid solution in the presence of palladium–calcium carbonate resulted in the consumption of two moles of hydrogen the reduction product (a mixture) gave cholestanone-4 on Oppenauer oxidation

is therefore considered to be that of Δ^5 cholestenone-4 (XXXIX), similarly, *iso* Δ^1-pregnenedione may be regarded as Δ^5 pregnenedione-4,20 The probable mechanism involved in the formation of the *iso* Δ^1 ketone is indicated in Chart 5 (only rings A and B are shown) The initial step is believed to involve a shift of the bromine atom from position 2 to position 4 (XXXIV to XXXV), this shift may serve to explain the formation of progesterone (V) in addition to Δ^1 allopregnenedione 3 20 (XXXII) from 2-bromoallopregnanedione-3 20 (XXXI) (see Chart 4) The bromine atom at carbon 4 in XXXV is replaced by a hydroxyl group and the ketol, XXXVI, thus formed undergoes rearrangement to XXXVII Dehydration of XXXVII to furnish XXXIX may occur via XXXVIII, XXXVIII resembles the so called *allo* cholesterol (XL) prepared by treating the toluenesulfonic ester of cholesterol (I) with potassium acetate in acetic anhydride

CHART 5

PROBABLE MECHANISM IN THE CONVERSION OF 2 BROMOCHOLESTANONE 3 (XXXIV) TO *iso* Δ^1 CHOLESTENONE (XXXIX)* (AND OF XXXI TO XXXIII)



* Only Rings A and B are shown

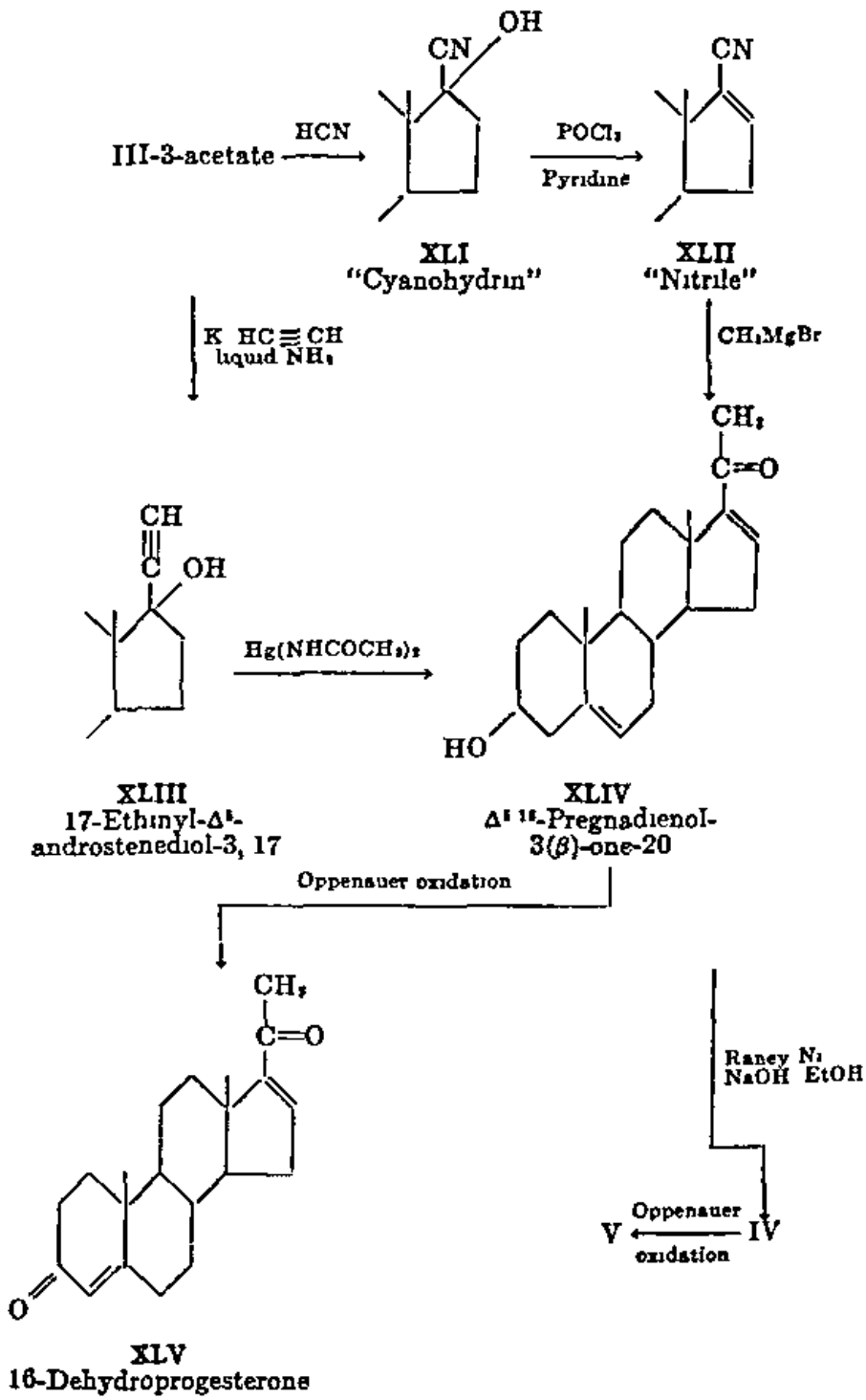
6. *From Dehydroisoandrosterone*

Several routes may be followed in preparing progesterone (V) from dehydroisoandrosterone (III) but the route used by Butenandt and

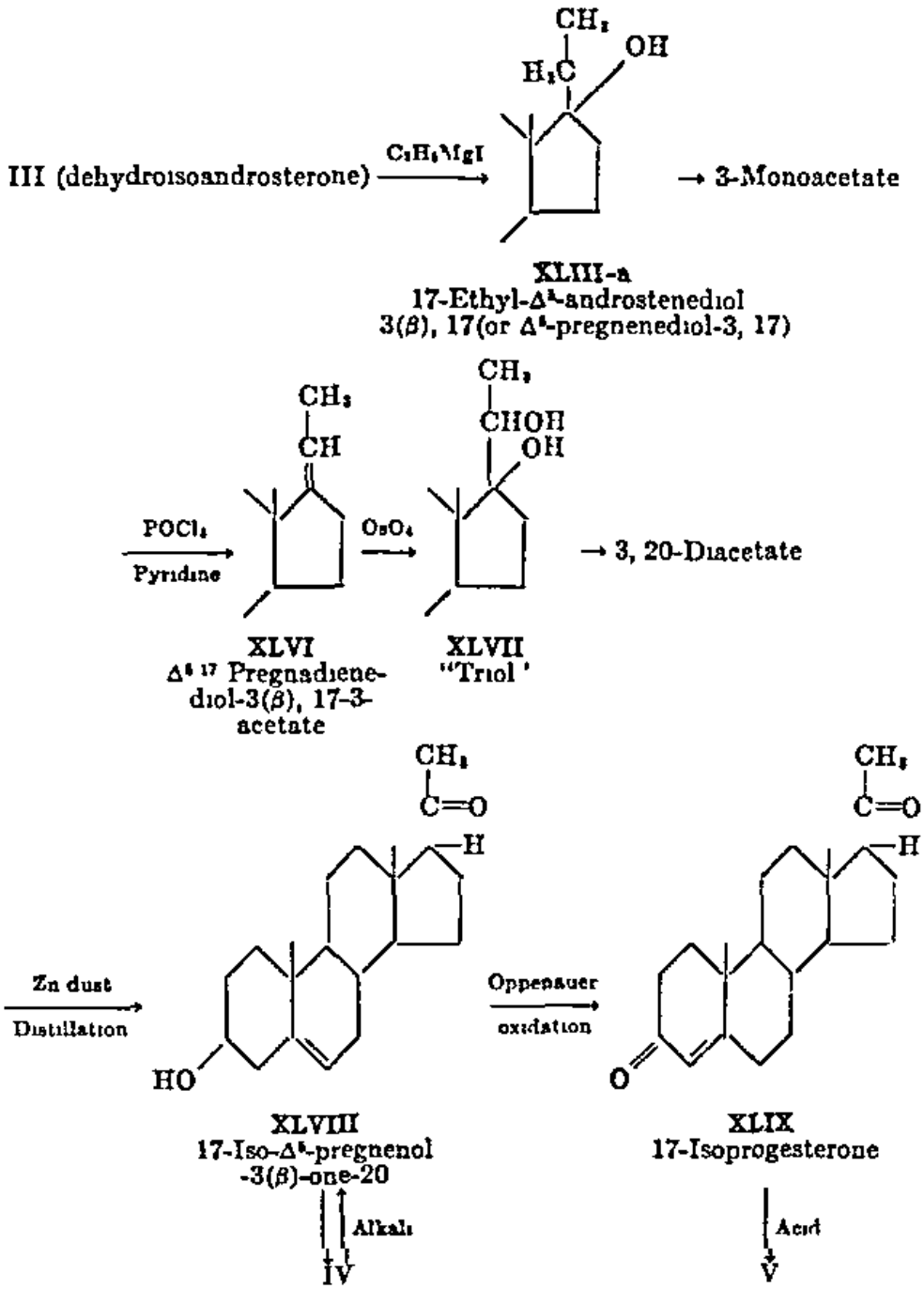
CHART 6

PARTIAL SYNTHESIS OF PROGESTERONE (V) FROM DEHYDROISOANDROSTERONE (III)

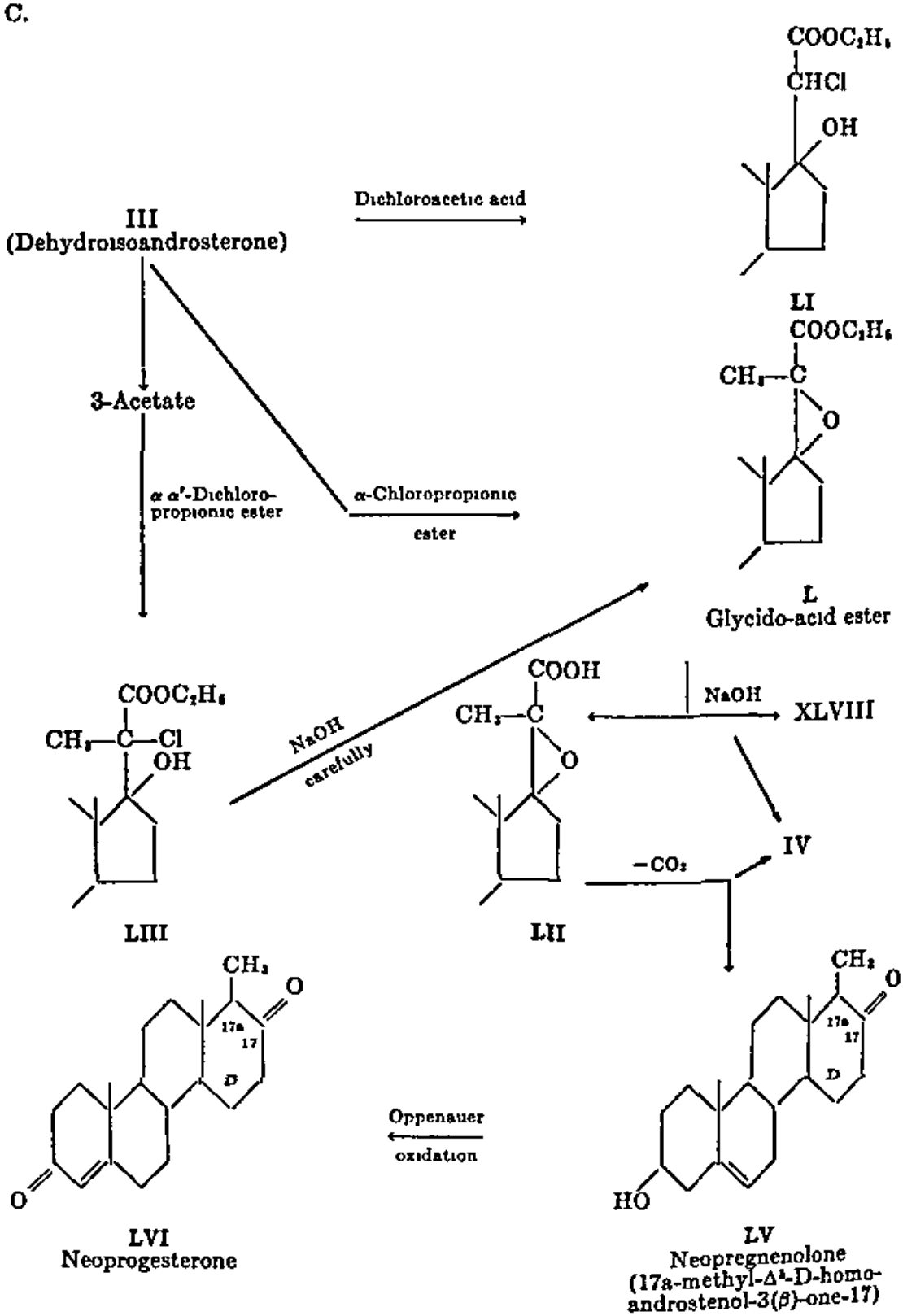
A.



B.


III (dehydroisoandrosterone)
C_2H_5MgI
3-Monoacetate
XLIII-a
17-Ethyl-Δ^5-androstenediol
3(β), 17(or Δ^5-pregnenediol-3, 17)
$POCl_3$
Pyridine
OsO_4
3, 20-Diacetate
XLVI
$\Delta^{5,17}$ Pregnadiene-diol-3(β), 17-3-acetate
XLVII
"Triol"
Zn dust
Distillation
Oppenauer
oxidation
XLVIII
17-Iso-Δ^5-pregnenol
-3(β)-one-20
Alkali
IV
XLIX
17-Isoprogesterone
Acid
V

C.


III
(Dehydroisoandrosterone)
Dichloroacetic acid
3-Acetate
α α'-Dichloro-propionic ester
α-Chloropropionic ester
LI
L
Glycido-acid ester
LIII
LII
NaOH carefully
NaOH
XLVIII
IV
−CO₂
Oppenauer oxidation
LVI
Neoprogesterone
LV
Neopregnenolone
(17a-methyl-Δ⁵-D-homo-androstenol-3(β)-one-17)

Schmidt-Thomé (32) is probably the most expedient (see Chart 6) The authors reacted dehydroisoandrosterone (III) acetate with hydrogen cyanide, thereby obtaining the cyanohydrin, XLI XLI, on treatment with phosphorus oxychloride and pyridine, gave the unsaturated nitrile, XLII XLII was treated with methyl magnesium bromide in order to obtain $\Delta^{5\ 16}$-pregnadienol-3(β) one-20 (XLIV) The Δ^{16} double bond in XLIV may be selectively reduced by treating an ethanolic solution of this compound with sodium hydroxide and Raney nickel, this results in the formation of Δ^5-pregnenol-3(β)-one-20 (IV) IV is readily converted to progesterone (V) by a modified Oppenauer reaction in which aluminum isopropoxide and cyclohexanone are employed (Oppenauer, Inhoffen *et al*, 171) The yield of progesterone (V) from dehydroisoandrosterone (III) is approximately 40%

The intermediate compound, XLIV, in the foregoing scheme may be obtained from dehydroisoandrosterone (III) by another way The acetyl derivative of III is treated with the potassium salt of acetylene in liquid ammonia to give the addition product, 17-ethinyl-Δ^5-androstenediol-3(β),7 (XLIII) (Kathol *et al*, 94, Ruzicka and Hofmann 186) XLIII, on reaction with the mercury salt of acetamide, forms XLIV in good yield (Goldberg and Aeschbacher, 67), the mechanism of the reaction wherein the side chain in XLIII-a undergoes hydration and dehydration, is not well understood

Another approach to the partial synthesis of progesterone (V) has been described by Butenandt and co-workers Dehydroisoandrosterone (III) is reacted with ethyl magnesium iodide to give Δ^5-pregnenediol-3(β),17 (XLIIIa) (Butenandt *et al*, 18) The hydroxyl group on carbon 3 in XLIIIa is protected by acetylation and the derivative is treated with phosphorus oxychloride and pyridine to give $\Delta^{5\ 17}$-pregnadienediol-3(β),3 acetate (XLVI) (Butenandt *et al*, 33) XLVI is converted to the triol, XLVII, with the aid of osmium tetroxide Distillation of the 3,20-diacetate of XLVII in the presence of zinc dust gave 17-iso-Δ^5-pregnenol-3(β)-one-20 (XLVIII), a substance which was found to be identical with the product obtained by alkali isomerization of Δ^5-pregnenol-3(β)-one-20 (IV) (Butenandt and Fleischer, 21) Oxidation of XLVIII by the method of Oppenauer results in the formation of 17 isoprogesterone (XLIX), it is readily isomerized to progesterone (V) with the aid of acid

The conversion of dehydroisoandrosterone (III) to Δ^5-pregnenol-3(β)-one-20 (IV) may also be achieved by application of the Darzens reaction Thus, Yarnall and Wallis (232) reacted dehydroisoandrosterone (III) with α chloropropionic ester, condensation occurred under the influence of sodium ethylate to give an oxidoester, L, which, on

treatment with sodium hydroxide, yielded a mixture of the free oxido-acid, LII, Δ^5-pregnenol-3(β)-one-20 (IV) and 17-iso-Δ^5 pregnenol-3(β)-one-20 (XLVIII) Optimum conditions for these reactions were determined by employing as model substances cyclopentanone and cyclohexanone Ercoli and Mamoli (55) substituted dichloroacetic acid in the initial step described above and obtained LI Miescher and Kagi (164), in similar studies, protected the hydroxyl group in dehydroisoandrosterone (III) by acetylation and then condensed the derivative with α,α'-dichloropropionic ester under the influence of magnesium amalgam The reaction product, LIII, on careful treatment with methanolic sodium hydroxide, yielded a mixture of the acetyl derivatives of the isomeric glycido esters of type L, further treatment of L with alkali gave the corresponding acids, LII Decarboxylation of LII was effected by heating the acetylated esters with quinoline, a mixture of Δ^5-pregnenol-3(β)-one 20 (IV) and neopregnenolone (LV) was obtained In contradistinction to 17 isopregnenolone (XLVIII), LVI is digitonin precipitable LV may be converted to neoprogesterone (LVI) by Oppenauer oxidation, the latter compound is not identical with 17-iso-progesterone (XLIX) In an effort to elucidate the structure of LVI, Ruzicka and Meldahl (188) prepared this compound by treating Δ^5 pregnenediol-3(β),17(α)-one-20 with phosphorus tribromide and dehalogenating the 17-bromo derivative thus obtained with zinc dust in acetic acid solution It was suspected that an internal rearrangement had occurred in the course of the formation of neopregnenolone (LV) Indeed, LV yielded on selenium dehydrogenation not a cyclopentenophenanthrene derivative but instead 1-methylchrysene (Ruzicka and Meldahl, 189) Neopregnenolone (LV) is consequently represented as the perhydrochrysene derivative, LV, which is designated as 17a-methyl-Δ^5 D-homoandrostenol-3(β)-one-17, (*i e*, ring D is no longer five-membered but is six membered), neoprogesterone is represented by LVI The 17-hydroxy-20 ketosteroids may be induced to undergo enlargement in ring D by more direct means, *e g*, by treatment with acid, and alkali, or more simply in certain instances by contact with alumina as in chromatographic analysis (for a more extensive discussion see Chapter XIII)

B Chemical Reactions of Progesterone and Related Products

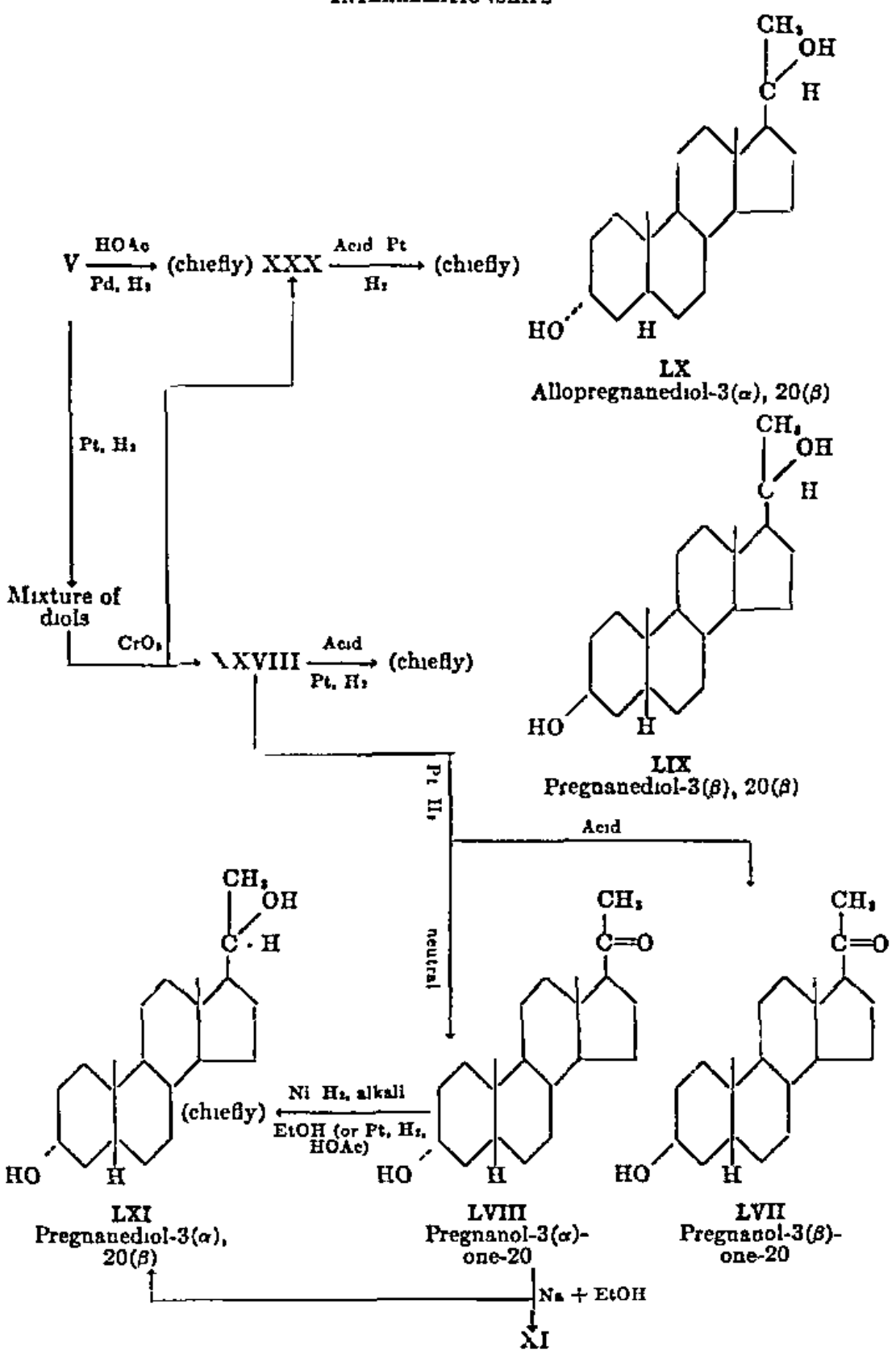
1 *Reduction*[5]

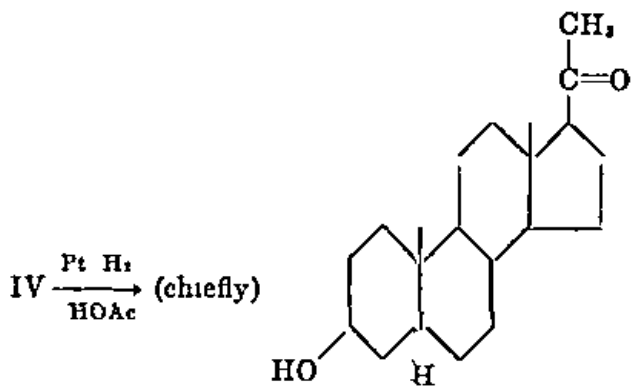
Although cholestenone, on catalytic hydrogenation, yields exclusively a product in which rings A and B are in a *cis* spatial relationship (Grasshof 68), it is not possible to make generalizations as to the stereochemical

[5] See Chart 7

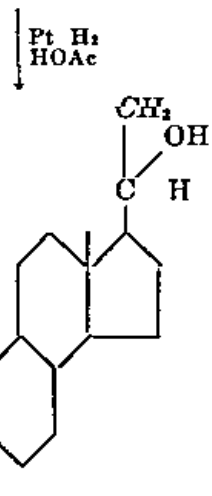
CHART 7

PROGESTERONE (V), PREGNANOLONES AND PREGNANEDIOLS. CHEMICAL INTERRELATIONSHIPS



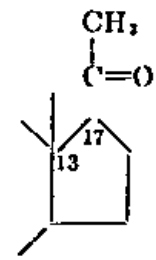


LXII
Allopregnanol 3(β)-one-20

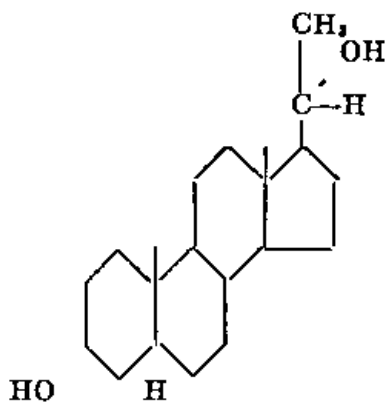


LXIII
Allopregnanediol-3(β), 20(β)

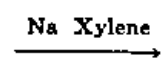


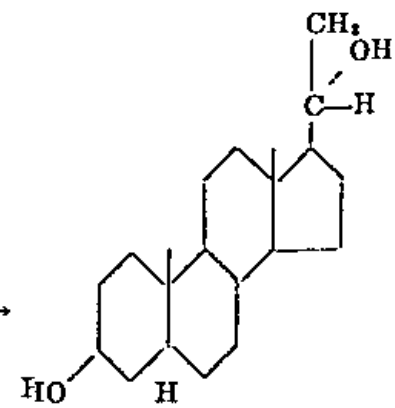


LXVI
"17-Iso-form"



LXIV
Allopregnanediol 3(α), 20(α)





LXV
Allopregnanediol 3(β), 20(α)

LIX $\xrightarrow{\text{Na Xylene}}$ LXI

course followed during the catalytic hydrogenation of other steroids of the 3 keto Δ^4 type In the case of progesterone (V), Butenandt and Fleischer (20) found that this substance, on hydrogenation in the presence of platinum, gave a mixture of diols which, on oxidation, yielded pregnanedione-3,20 (XXVIII) (rings A and B *cis*) and allopregnanedione-3,20 (XXX) (rings A and B *trans*), XXX was present in largest proportion As Shoppee and Reichstein (202) have summarily pointed out, the nature of the substituent in the progesterone molecule greatly influences the course of the reduction Thus, 21-hydroxyprogesterone (desoxycorticosterone) (LXL) yields on reduction chiefly a product of the *cis* configuration with respect to rings A and B (Wettstein and Hunziker, 228), whereas 11,21-dihydroxyprogesterone (corticosterone) (LXLa) yields chiefly the *trans* variety (Steiger and Reichstein, 117) The nature of the reduction products derived from 3 keto Δ^4-steroids of the C_{27} C_{24} C_{22}, C_{21}, C_{20}, and C_{19} series is briefly indicated in Table I

The stereochemical course followed during the catalytic reduction of the 3 keto saturated compounds of the pregnane or allopregnane series conforms roughly with the von Auwers-Skita rule[6] (215), *i e* reduction in an acid medium usually gives *cis* forms (with respect to the spatial relationship of the hydroxyl group on carbon 3 with the hydrogen atom on carbon 5), whereas *trans* compounds are formed in a neutral medium Thus Marker and Lawson (131) found that hydrogenation of allopregnanedione-3,20 (XXX) in an acetic–hydrobromic acid solution in the presence of Adams' catalyst gave allopregnanediol 3(α),20(β)(LX), a small amount of allopregnanediol-3(β),20(β) (LXIII) was also formed Reduction of pregnanedione-3,20 under the same conditions gave pregnanediol-3(β),20(β) (LIX) almost exclusively Partial catalytic hydrogenation of pregnanedione-3,20 (XXVIII) in a neutral solution of alcohol yielded pregnanol-3(α) one-20 (LVIII), whereas partial reduction of XXVIII in an acetic–hydrobromic acid solution yielded pregnanol 3(β)-one 20 (LVII) (Marker *et al*, 126) It is noteworthy that the nature of the medium does not appreciably influence the stereochemical course followed during the catalytic hydrogenation of the carbonyl group on carbon 20 Thus Marker and Lawson (131) obtained only 20(β)-hydroxy derivatives on catalytic reduction of either pregnanedione-3,20 (XXVIII) or of allopregnanedione-3,20 (XXX) in an acid medium, catalytic reduction of progesterone (V) in a neutral alcoholic solution similarly yielded 20(β)-hydroxy steroids Meystre and Miescher (162) hydrogenated pregnanol-3(α) one 20 (LVIII) in an alkaline ethanol solution in the presence of nickel and obtained pregnanediol 3(α),20(β) (LXI), a small

[6] This generalization is based on hydrogenation studies on simple nonsteroid compounds such as alkylated cyclohexanone derivatives

TABLE I

CATALYTIC HYDROGENATION OF 3 KETO Δ^4 STEROIDS[a]

Steroid hydrogenated	Proportion of *cis* and *trans* products (Rings A B)	Investigator
Cholestenone	*Cis* (only)	Grasshof (68)
Bile acids and derivatives		
3-Keto Δ^4-cholenic acid	Chiefly *cis* little *trans*	Schoenheimer and Berliner (196)
3 Keto Δ^4-bisnorcholenic acid	Chiefly *cis* much *trans*	Butenandt and Mamoli (25)
3 11 Diketo Δ^4-etiochol enic acid	Chiefly *trans*	Mason *et al* (154) Steiger and Reichstein (209)
3 12 Diketo Δ^4 cholenic acid	Chiefly *cis* little *trans*	Sawlewicz and Reichstein (194)
Progesterone (V)	Chiefly *trans* some *cis*	Butenandt and Fleischer (20)
	Trans and *cis* mixture	Marker and Lawson (131)
	Chiefly *trans*	Pearlman (172)
Progesterone derivatives		
16-Dehydroprogesterone (XLV)	*Cis* (only)	Marker *et al* (143)
9 Dehydroprogesterone (CX)	Chiefly *trans* little *cis*	Shoppee and Reichstein (202)
21 Hydroxyprogesterone (LXL)	Chiefly *cis* little *trans*	Wettstein and Hunziker (228)
Corticosterone (LXLa)	Chiefly *trans*	Steiger and Reichstein (209)
Testosterone (and 17 keto steroids)		
Testosterone (CXXI)	*Trans* (only)	Butenandt *et al* (34)
Adrenosterone	Chiefly *trans*	Steiger and Reichstein (208)

[a] Steric relationship between rings A and B in reduction products

amount of pregnanediol 3(α) 20(α) (XI) was also isolated if the hydrogenation is carried out in acetic acid solution in the presence of platinum oxide the same products are obtained but the quantity of LXI formed is considerably reduced Reduction of 20 keto compounds of the pregnane (or allopregnane) series with sodium and alcohol favors the formation of 20(α) hydroxy derivatives (Butenandt and Müller 28) In the experi

ments described by Meystre and Miescher (162), reduction of pregnanol-3(α)-one-20 (LVIII) with sodium and ethanol yielded about twice as much pregnanediol-3(α),20(α) (XI) as pregnanediol 3(α),20(β) (LXI) The designation α or β with reference to the configuration of the hydroxyl group on carbon 20 is purely arbitrary[7], only pregnanediols of the 20(α) variety have thus far been isolated from urinary sources (see Section II)

Partial hydrogenation of Δ^5 pregnenol-3(β)-one-20,3-acetate (IV) in acetic acid solution in the presence of platinum oxide yields chiefly allopregnanol-3(β)-one-20,3-acetate (LXII) and very little pregnanol-3(β)-one-20,3 acetate (LVII) (Plattner *et al*, 178) Complete hydrogenation of Δ^5 pregnenol-3(β)-one-20 (IV) under the same conditions yields allopregnanediol-3(β),20(β) (LXIII) (Pearlman, 172) The course followed during the reduction of the double bond in ring B in IV parallels that followed during the reduction of the analogous compound, cholesterol, I, to dihydrocholesterol (rings A and B *trans*) (Willstätter and Mayer, 229)

2 *Stereoisomerization*[8]

Treatment of allopregnanediol-3(α),20(α) (LXIV) and of pregnanediol-3(β),20(β) (LIX) with sodium in boiling xylene yield, respectively, allopregnanediol-3(β),20(α) (LXV) and pregnanediol-3(α) 20(β) (LXI) (Marker *et al*, 127) The net effect in each instance is the formation of a steroid possessing a hydroxyl group on carbon 3 in *trans* spatial relationship with the hydrogen atom on carbon 5, the configuration of the hydroxyl group on carbon 20 is not affected Windaus (230) had previously employed similar means to induce epimerization of the cholestanols

The C_{17} side chain in 20 ketopregnane compounds can be induced, under the influence of alkali, to undergo a change in spatial configuration Thus, for example, Butenandt and Mamoli (23) found that allopregnanol 3(β)-one-20 (LXII) is converted, to a certain extent, to 17 isoallopregnanol-3(β)-one-20 (LXVI) on treatment with alkali, the change is a reversible one, and acid catalysts favor the formation of the normal (or naturally occurring) compound In the naturally occurring compounds such as progesterone and related products, the side chain at C_{17} is believed to possess the β configuration, *i e*, the side chain is in *a cis* spatial relationship with respect to the angular methyl group attached to carbon 13 (see Chapter XIII for a fuller discussion) Both allopregnanol-3(β)-one-20 and its 17 stereoisomeride are precipitated by digitonin However, 17-iso-Δ^5-pregnenol-3(β)-one-20 (XLVIII) is not precipitated by digitonin

[7] This type of stereoisomerism is not geometrical in character (fixed position above or below the plane of the ring), but is instead of the classical tartaric acid type (free rotation *i e*, the C_{17} side chain in pregnanediol, etc is free to rotate)

[8] See Chart 7

(Butenandt and Fleischer, 21) It is curious that allopregnanol-3(β)-one 20,3 acetate (LXII) and allopregnanedione-3,20 (XXX) (but not the iso compounds) very slowly form insoluble digitonides (Butenandt and Mamoli, 23) This is an exception to the rule that acetyl derivatives and ketones in the steroid series do not form stable molecular compounds with digitonin

3 *Relative Reactivity of Functional Groups*

The carbonyl group at carbon 3 is more reactive than one at carbon 20 in compounds of the pregnane or allopregnane series, *e g*, partial hydrogenation of pregnanedione-3,20 (XXVIII) results in the formation of pregnanol-3-one-20 (LVII or LVIII) (Marker *et al*, 126) In pregnanediol-3(α),20(α) (XI) the hydroxyl group on carbon 3 may be preferentially acetylated (Hirschmann, 82) by heating the diol in glacial acetic acid, the 20-monoacetate as well as the 3,20 diacetate of XI are formed to some extent and may be removed by chromatography Conversely, partial hydrolysis of XI-3 20-diacetate gives the 20 monoacetyl derivative (Butenandt and Schmidt, 30) The hydroxyl group on carbon 3 in XI may be oxidized preferentially by the Oppenauer method if the reaction is carried out at 40°C (unpublished observations cited by Gallagher and Xenos 39)

4 *Degradation to 17-Ketosteroids (C_{19} Steroids)*[9]

It is possible to convert 20 ketopregnane (or allopregnane) compounds to the corresponding 17-ketosteroids by treatment, *e g*, of the 20 ketone, LXII, with methyl magnesium iodide to obtain the dimethylcarbinol, LXVII, followed by dehydration of LXVII by heating in a mixture of acetic anhydride and acetic acid and ozonolysis of the unsaturated product LXVIII (Butenandt and Cobler, 17, Butenandt and Muller, 28) The position of the double bond in LXVIII is apparently between carbon atoms 17 and 20 Koechlin and Reichstein (100) repeated and extended the experiments of Butenandt and Cobler, employing LXII, in addition to LXVIII, substances isomeric with it were obtained One of these isomers LXX, contains a double bond between carbon atoms 20 and 22 (or 21) since it yields LXII on ozonolysis, the structure of the other isomer could not be determined The type of double bond isomer obtained in these studies is dependent on the nature of the dehydrating agent employed Marker *et al* (123) converted allopregnanol 20(β)-one-3 to androstanedione 3,17 by heating the starting product with zinc chloride and acetic acid and submitting the unsaturated product thus obtained to ozonolysis the intermediate unsaturated product was not isolated

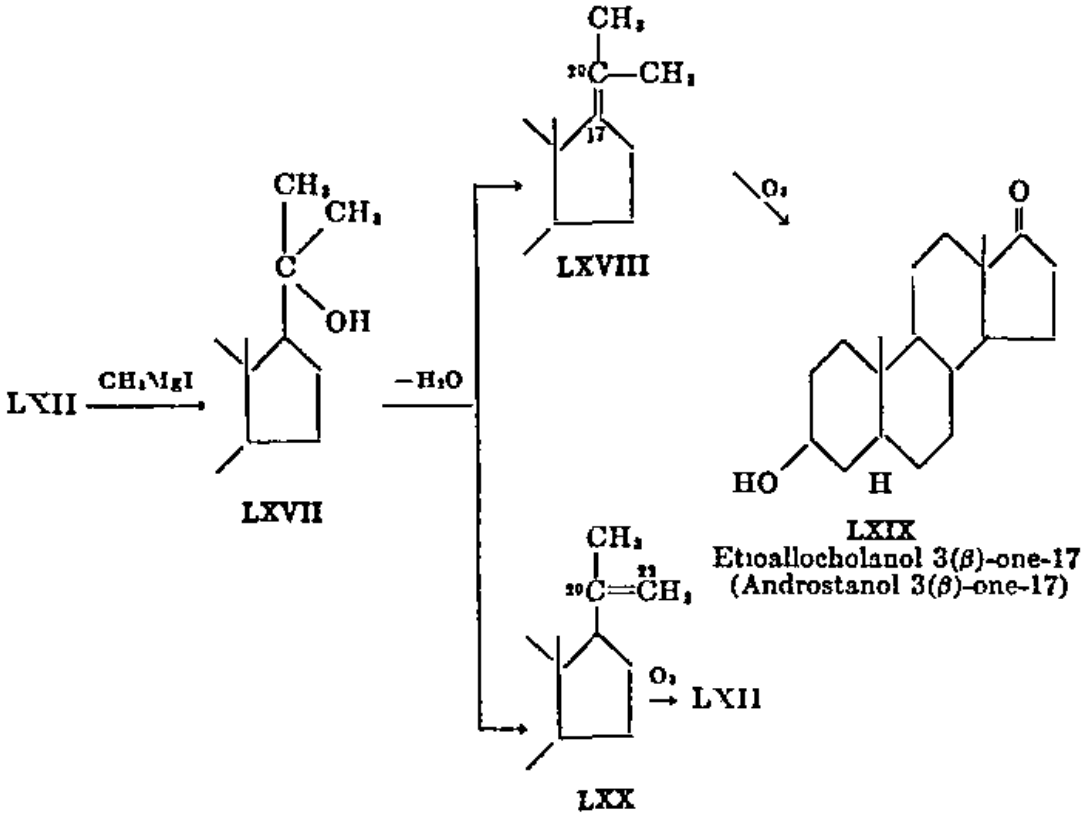
[9] See Chart 8

Hirschmann (82) converted XI-3 monoacetate to its 20-toluenesulfonyl derivative, LXXI, LXXI, on treatment with pyridine followed by hydrolysis, gave Δ^{17}-pregnenol-3(α) (LXXII) in excellent yield This product was hydroxylated with the aid of osmium tetroxide to give a pregnanetriol-3(α),17,20 (LXXIII) isomeric with the triols isolated from

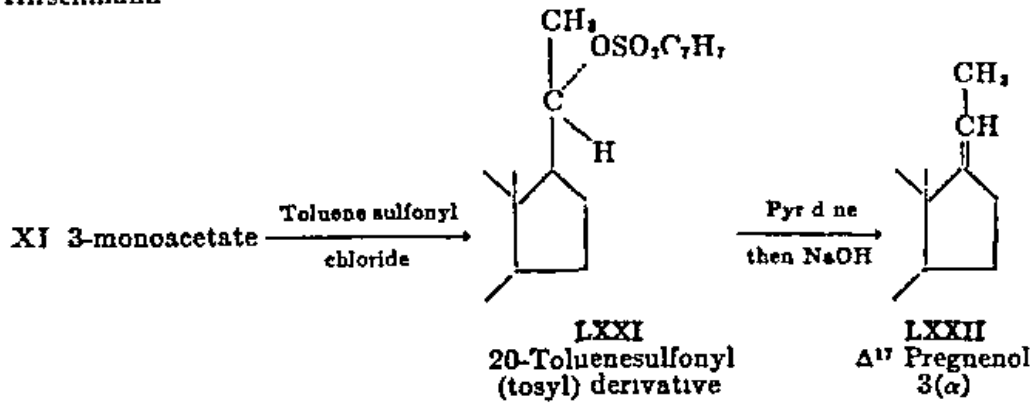
CHART 8

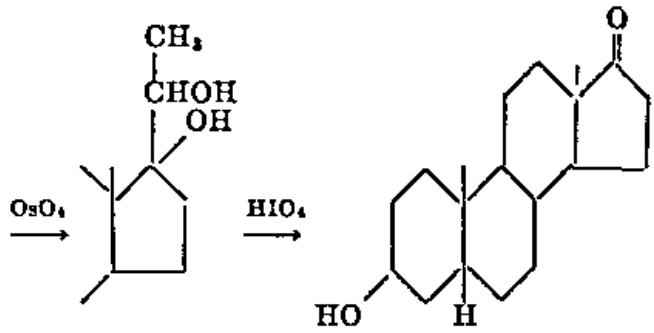
DEGRADATION OF C21 STEROIDS TO 17 KETOSTEROIDS (C19-STEROIDS)

A Butenandt



B Hirschmann

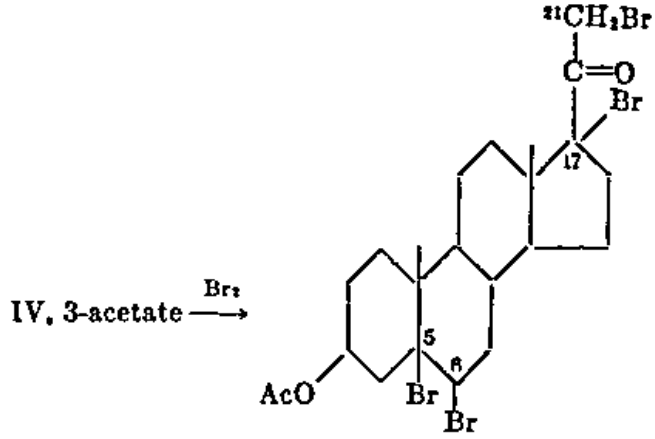




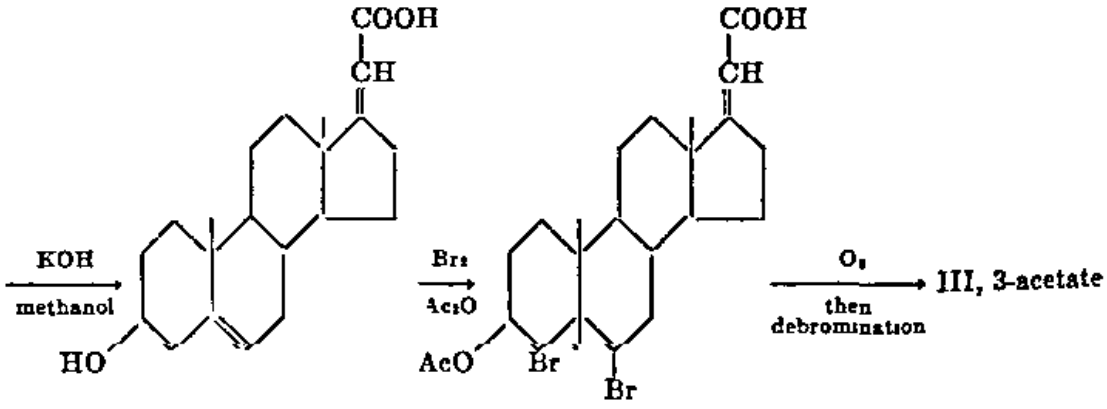
LXXIII
Pregnanetriol

LXXIV
Etiocholanol-3(α)-one-17

C Marker



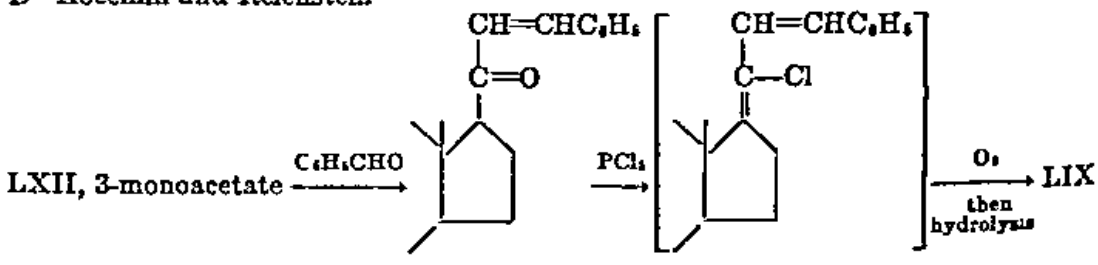
LXXV
5, 6, 17, 21-Tetrabromo derivative



LXXVI

LXXVII

D Koechlin and Reichstein



LXXVIII

LXXIX

natural sources It was readily converted to etiocholanol-3(α)-one-17 (LXXIV) by oxidation with periodic acid

Marker and co-workers (113) converted Δ^5-pregnenol-3(β)-one-20 (IV) to dehydroisoandrosterone (III) by the method of Butenandt *et al* (17,28), as described above, it was necessary to protect the 5,6 double bond in the intermediary $\Delta^{5,17}$-diene by preferential bromination Another route followed by Marker (113) is forthwith described IV-3-monoacetate was brominated to give the 5,6 17,21 tetrabromo derivative (LXXV) in good yield, LXXV on treatment with methanolic potassium hydroxide yielded the corresponding $\Delta^{5,17}$-pregnadienoic acid 21 (LXXVI), the 5,6 double bond in LXXVI was preferentially brominated in acetic anhydride and the 17,20 double bond in LXXVII exposed to the action of ozone, the oxidation product, on debromination gave III-3 acetate Marker *et al* (116,117) similarly converted saturated 20 ketopregnane compounds to 17-ketoetiocholane derivatives Koechlin and Reichstein (100) repeated and extended these studies to include pregnanediol-3(α),12(α)-one-20 (XXII), the over-all yield of 17-ketosteroid was about 7%

Direct oxidative procedures may also be employed to eliminate the C_{17} side chain of pregnane compounds Hoehn and Mason (85) treated XXII with chromic acid and isolated the corresponding 17-ketosteroid Marker *et al* (109,110,142,145) treated 20 ketopregnane and 20 ketoallopregnane compounds with persulfuric acid and obtained the corresponding 17-ketosteroids, LIX and LXXIV (as the acetates) as well as other oxidation products (*vide infra*)

Koechlin and Reichstein (100) prepared 21-benzalallopregnanol-3(β)-one-20,3-acetate (LXXVIII) (a derivative first described by Marker and Wittle, 148), and treated it with one mole phosphorus pentachloride in benzene to obtain a product LXXIX, which is probably an enol chloride, LXXIX was subjected to ozonolysis and then hydrolysis to obtain LIX in approximately 45% yield Yet, when this degradative process was applied to XXII, no 17-ketosteroid could be isolated

5 *Degradation to Etiocholanic Acids (C_{20} Steroids)*[10]

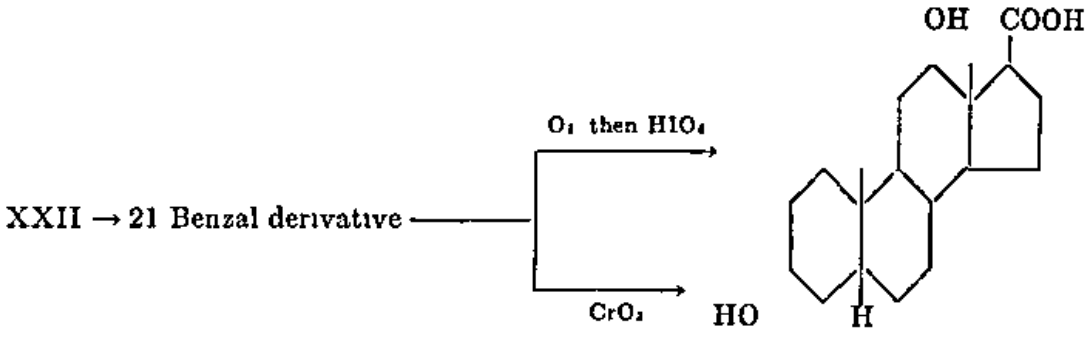
Hoehn and Mason (85) degraded desoxycholic acid (XVI) to the corresponding 20 ketopregnane, XXII, and condensed XXII with benzaldehyde, thereby obtaining the 21-benzal derivative, LXXVIII LXXVIII, on ozonolysis and periodic oxidation, yielded the corresponding etiocholanic acid, LXXX Marker and Wittle (148) succeeded in preparing the 21 benzal derivatives of allopregnanol-3(β)-one-20 (LXII), pregnanol-3(β)-one-20 (LVII), and of pregnanol-3(α)-one-20 (LVIII) in

[10] See Chart 9

CHART 9

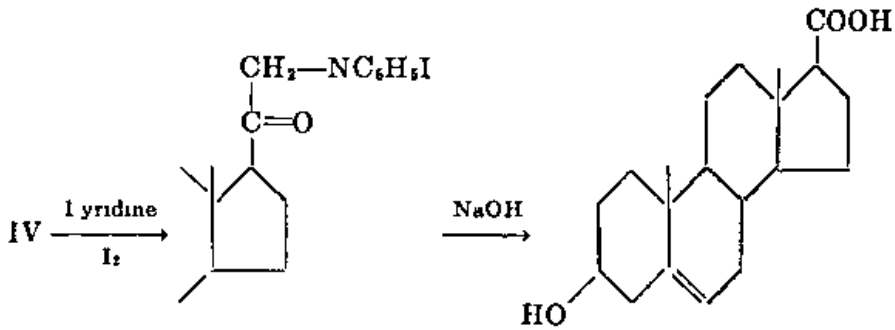
DEGRADATION OF C_{21} STEROIDS TO ETIOCHOLANIC ACIDS (C_{19}-STEROIDS)

A Hoehn and Mason Marker



LXXX
3(α), 12(α)-Dihydroxy-etiocholanic acid

B King



LXXXI

LXXXII
3(β)Hydroxy-Δ⁵ etiocholenic acid

excellent yield, chromic acid oxidation of the acetyl derivatives gave the respective etiocholanic acids in approximately 70% yields Etiocholanic acids may also be obtained as a by-product in the oxidation of 20-keto-allopregnane compounds with persulfuric acid (Marker, 142) (also Marker and Turner, 145) (*vide infra*) King (96) has described a simple scheme for converting Δ⁵-pregnenol-3(β) one-20 (IV) to 3(β)-hydroxy-Δ⁵-etiocholenic acid (LXXXII), IV, on treatment with iodine and pyridine is converted to the pyridinium iodine derivative, LXXXI, which is decomposed with sodium hydroxide, the yields are claimed to be very good

6 *Conversion to 21-Hydroxylated Derivatives*[11]

Following the method of Baeyer and Villiger (7), Marker (109) and also Marker and Turner (145) oxidized 20-ketopregnane compounds with persulfuric acid and obtained the corresponding 21-hydroxylated deriva-

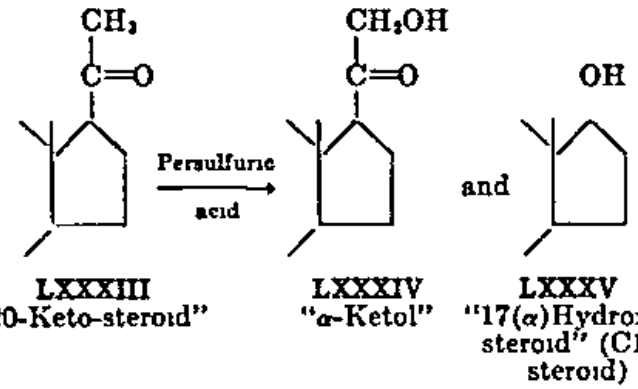
[11] See Chart 10

tives (as well as other oxidation products) (LXXXIII → LXXXIV + LXXXV). Koechlin and Reichstein (100) repeated this study, employing allopregnanol-3(β)-one-20,3-acetate (LXII), but found the yields of the desired oxidation products to be very poor Another route for the preparation of compounds of type LXXXIV was described by Marker *et al* (115). Pregnanol-3(β)-one-20 (LVII) was brominated in acetic acid solution to give the 17,21-dibromide LXXXVI in high yield Gentle treatment of LXXXVI with potassium acetate and acetic

CHART 10

CONVERSION OF 20-KETOSTEROIDS (C_{21}-STEROIDS) TO C_{21}-OH DERIVATIVES

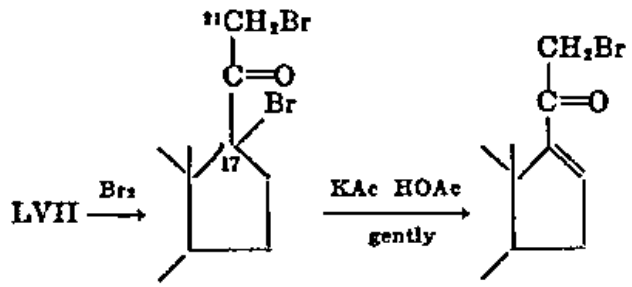
A. Marker



LXXXIII "20-Keto-steroid"

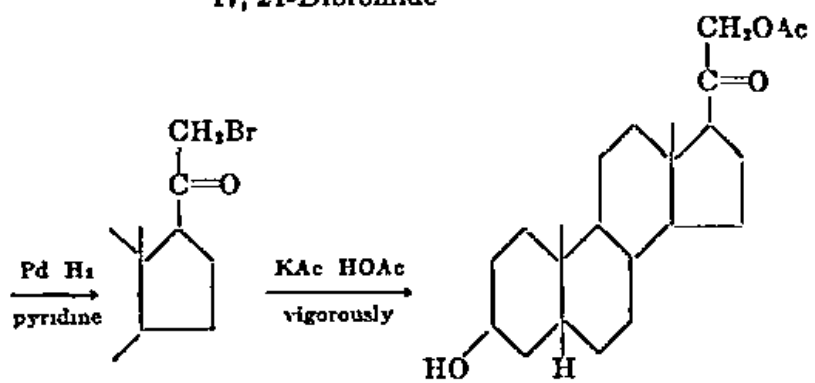
LXXXIV "α-Ketol"

LXXXV "17(α)Hydroxy-steroid" (C19-steroid)



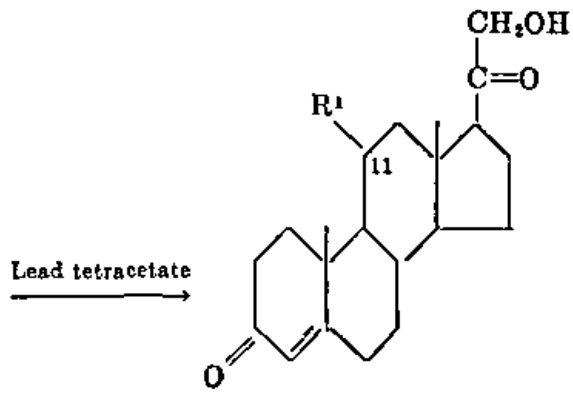
LXXXVI 17, 21-Dibromide

LXXXVII



LXXXVIII 21-Bromide

LXXXIX Pregnanediol-3(β),21-one-20-21-monoacetate



LXL
Desoxycorticosterone LXL
(R = H) Corticosterone
LXLa (R' = OH)

acid gave 21-bromo-Δ^{16}-pregnenol 3(β)-one-20 (LXXXVII), which was hydrogenated in pyridine solution in the presence of palladium to give 21-bromopregnanol 3(β) one 20 (LXXXVIII) Vigorous treatment of LXXXVIII with potassium acetate and acetic acid resulted in the formation of pregnanediol-3(β),21-one-20,21-monoacetate (LXXXIX) Plattner *et al* (178) applied the above mentioned preparative procedure (with certain changes in the sequence of steps) using as starting product allopregnanol 3(β)-one-20 3-acetate (LXII) LXII-3-acetate was converted to the corresponding 17,21-dibromide (first prepared by Marker, Crooks, and Wagner, 116, also described by Koechlin and Reichstein, 100) Gentle treatment of the 17,21-dibromide with acetic acid, acetic anhydride, and potassium acetate resulted in the elimination of bromine at carbon 17 and in the formation of a substance containing a double bond between carbon atoms 16 and 17 More vigorous treatment of the latter compound with the same reagents effected replacement of the bromine atom on carbon 21 with an acetyl group to give Δ^{16}-allopregnendiol-3(β) 21-one 20 3 21-diacetate, the Δ^{16}-double bond of this compound was then selectively hydrogenated

Progesterone (V) may be hydroxylated at carbon 21 by treatment with lead tetracetate but the yield of desoxycorticosterone (LXI) is very low (Erhart *et al* , 57)

7 *Miscellaneous Reactions*[12]

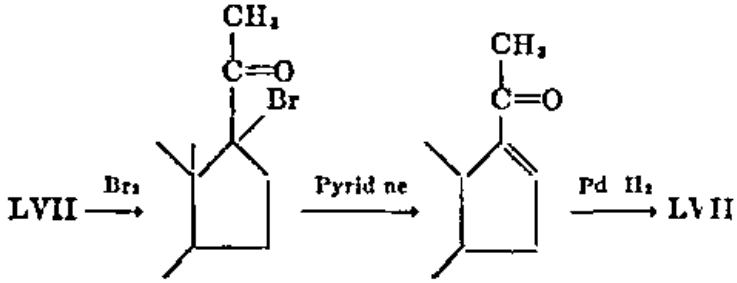
As a supplement to the bromination studies mentioned above, other reactions of this sort are described forthwith As a general rule, bromination of ketones results in the introduction of bromine in positions α to the carbonyl group Thus Butenandt and Mamoli (24) (also Masch, 24) monobrominated pregnanol-3(α)-one-20 (LVIII) and obtained the

[12] See Chart 11

corresponding 17 bromo derivative, the latter substance was dehalogenated and oxidized to give Δ^{16} pregnenedione-3 20 (IX) According to Marker (114) monobromination of pregnanol-3(β)-one-20 (LVII)

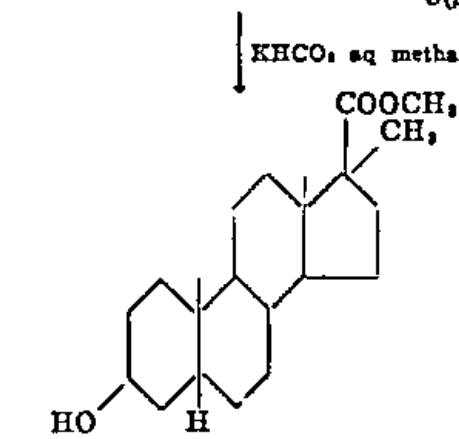
CHART 11

MISCELLANEOUS REACTIONS OF PREGNANE COMPOUNDS

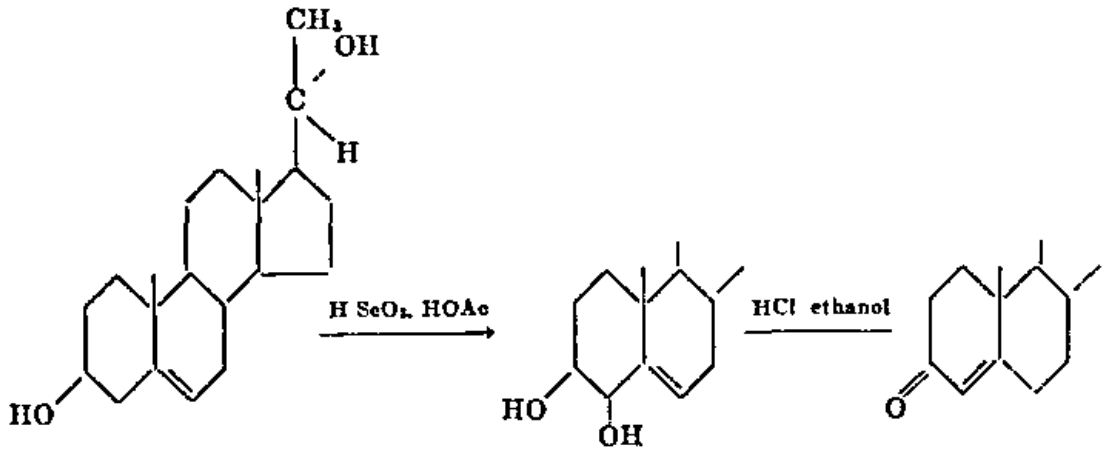


LXLI
17 Bromide

LXLII
Δ^{16}-Pregnenol
3(β)-one-20



LXLIII
3(β) Hydroxy 17 methyl-etiocholanic acid methyl ester



LXLIV
Δ^{5}-Pregnened ol
3(β) 20(α)

LXLV
Δ^{5} Pregnenetriol
3 4 20

LXLVI
Δ^{4}-Pregnenol
20(α)-one-3

results in the formation of the 17-bromide, LXLI, in high yield Treatment of LXLI with pyridine yields Δ^{16}-pregnenol-3(β)-one-20 (LXLII), which may be transformed to the starting product, LVII, on partial hydrogenation using palladium catalyst Position 17 is favored in these bromination reactions but further bromination results also in the introduction of the halogen at position 21 (see above) The bromination studies by Marker (114) were repeated by Koechlin and Reichstein (100) and extended to include pregnanediol-3,12-one-20 (XXII) as the starting product Plattner *et al* (179) prepared Δ^{16}-allopregnenol-3(β)-one-20,3 acetate via the 17-bromide in connection with the synthesis of 14-hydroxylated steroids A curious rearrangement of 17-bromopregnanol-3(β)-one 20 (LXLI) has been described by Marker and Wagner (77), LXLI when refluxed with aqueous methanolic potassium bicarbonate is converted to the methyl ester of 3(β)-hydroxy-17-methyletiocholanic acid (LXLIII) The reaction is analogous to that described by Aston and Greenburg (6) wherein α bromo secondary-alkyl ketones rearrange under the influence of sodium alcoholates to give esters of tertiary acids

Δ^5-Pregnenediol-3(β),20(α) (LXLIV) may be oxidized with selenious acid to the corresponding 3,4,20-triol, LXLV, which, on treatment with hydrochloric acid, yields Δ^4-pregnenol 20(α)-one-3, (Marker, 118) This type of reaction had been previously described for the analog cholesterol (I)[13] by Rosenheim and Starling (184)

Butenandt and Wolff (37) investigated the effect of ultraviolet light on $\alpha\beta$-unsaturated ketones, irradiation of a benzene or benzene-hexane solution of progesterone (V) resulted in the formation of a high melting bimolecular compound possessing only two reactive carbonyl groups

C Progesterone like Substances

1 *Oxygenated Derivatives of Progesterone*

Ehrenstein (45) and Ehrenstein and Stevens (48) prepared 6-ketoprogesterone (CIII) and 6(β) acetoxyprogesterone (CII)[14] by partial

[13] Cholesterol acetate on treatment with selenium dioxide yields not only Δ^1-cholestenediol-3 4 but also Δ^4-cholestenediol-3 6

[14] The designations previously assigned by Ehrenstein to the spatial configurations of the hydroxyl groups of the pregnenolone derivatives obtained by him are revised in the light of the recent studies on the corresponding derivatives of the analogous compound cholesterol (I) (see Reichstein and Reich 181, for a recent review on the latter subject) In line with the proposals by Reichstein and Reich (181) that the α-oxide of cholesterol be designated as 3(β) hydroxy 5 6(α)-oxidocholestane or more simply as 3(β) hydroxy 5 6-oxidocholestane (allocation of an index to the carbon 5

synthesis from Δ^5-pregnenol-3(β)-one-20 (IV) (see Chart 12 for an outline of the preparative routes) Chromic acid oxidation of IV yields allopregnanol-5 trione-3,6,20 (C)[15], dehydration of C with dry hydrochloric acid in chloroform solution gives CIII [16] It is of interest that 6-keto-progesterone (CIII) is biologically inactive The intermediate, C, may also be obtained by *chromic acid oxidation of either allopregnanetriol-3(β), 5,6(α)-one-20* (LXLVIII) or of allopregnanetriol-3(β),5 6(β)-one 20 (LXLIX) LXLVIII is obtained from IV by hydroxylation with osmium tetroxide LXLIX is obtained by treatment of the acetyl derivative of IV with hydrogen peroxide followed by hydrolysis It may also be prepared by treatment of IV with perbenzoic acid to obtain 5,6-oxidoallopregnanol-3(β)-one-20 (LXLVII), which, on heating with acetic acid, yields LXLIX-6 monoacetate, from which the free triol is obtained by hydrolysis Chromic acid treatment of LXLIX furnishes allopregnanediol 5 6(β)-dione-3 20,6 monoacetate (CI), if CI-6-monoacetate is treated

TABLE II

SOME PROGESTATIONALLY ACTIVE COMPOUNDS[a]

Compound	Investigator
3 Enolacetate[b] of progesterone (V)	Westphal (222)
6(β) Acetoxyprogesterone (CII)	Ehrenstein and Stevens (48)
21 Hydroxyprogesterone (LXL) (desoxycorticosterone)	Leathem and Crafts (102)
6-Dehydroprogesterone (CVIII)	Wettstein (226)
11 Dehydroprogesterone (CIX)	Hegner and Reichstein (79)
9 Dehydroprogesterone (CX)	Shoppee and Reichstein (202)
10 *Norprogesterone* (CXIX)	Ehrenstein (46)
Pregneninol 17-one 3 (CXX)	Inhoffen *et al* (92)
	Ruzicka *et al* (187)
17 Methyltestosterone (CXXII)	Cited by Ruzicka and Rosenberg (191)

[a] Other than progesterone only the more active compounds are listed

[b] Prepared by treating progesterone (V) with a mixture of acetic anhydride and acetyl chloride

position can be omitted since the spatial configuration of the 5-oxido group is already indicated by the term cholestane) it is suggested that the α-oxide derived from pregnenolone (IV) be called 5,6-oxidoallopregnanol 3(β)-one 20 (LXLVII) the β-oxide of pregnenolone has not as yet been described although the β-oxide of cholesterol *3(β)hydroxy-5 6-oxidocoprostane is known The oxidoderivative obtained by* Ehrenstein and Decker (47) on permanganate oxidation of Δ^5 pregnenol 3(β) one 20 3-monoacetate (IV) may possibly be the β-oxide form but this remains to be established Dr Ehrenstein and the reviewer concur in the revised steric formulations of the pregnenolone reaction products described in Chart 12

[15] The configuration of the 5 hydroxyl group, which is α is already indicated by the term allo the designation α may therefore be omitted

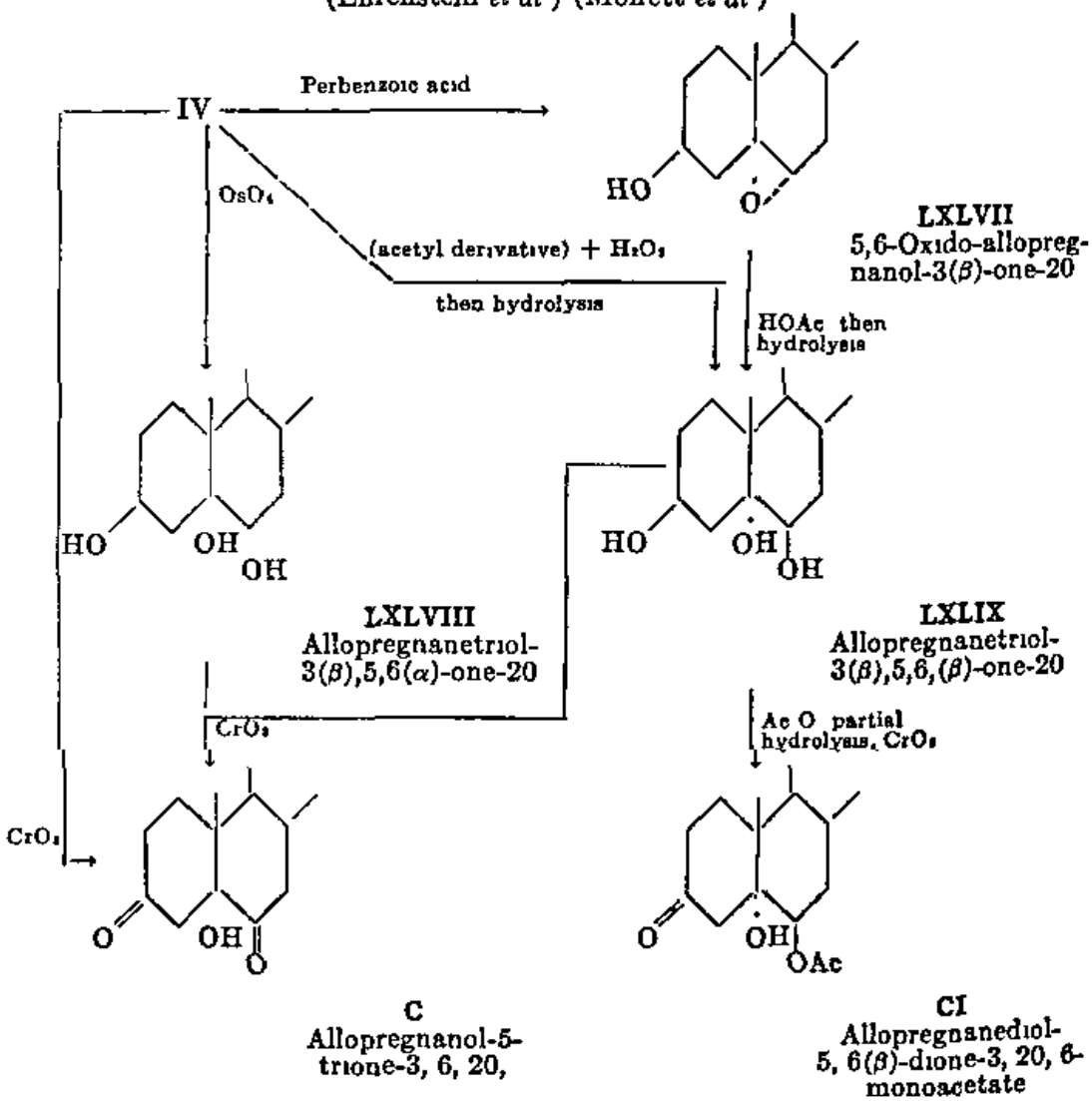
[16] 6 Ketoprogesterone (CIII) has recently been prepared by converting pregnane trione-3 6 20 (CVI) (derived from hyodesoxycholic acid) to the 4 bromo derivative and debrominating the latter in boiling pyridine (Moffett *et al* 88)

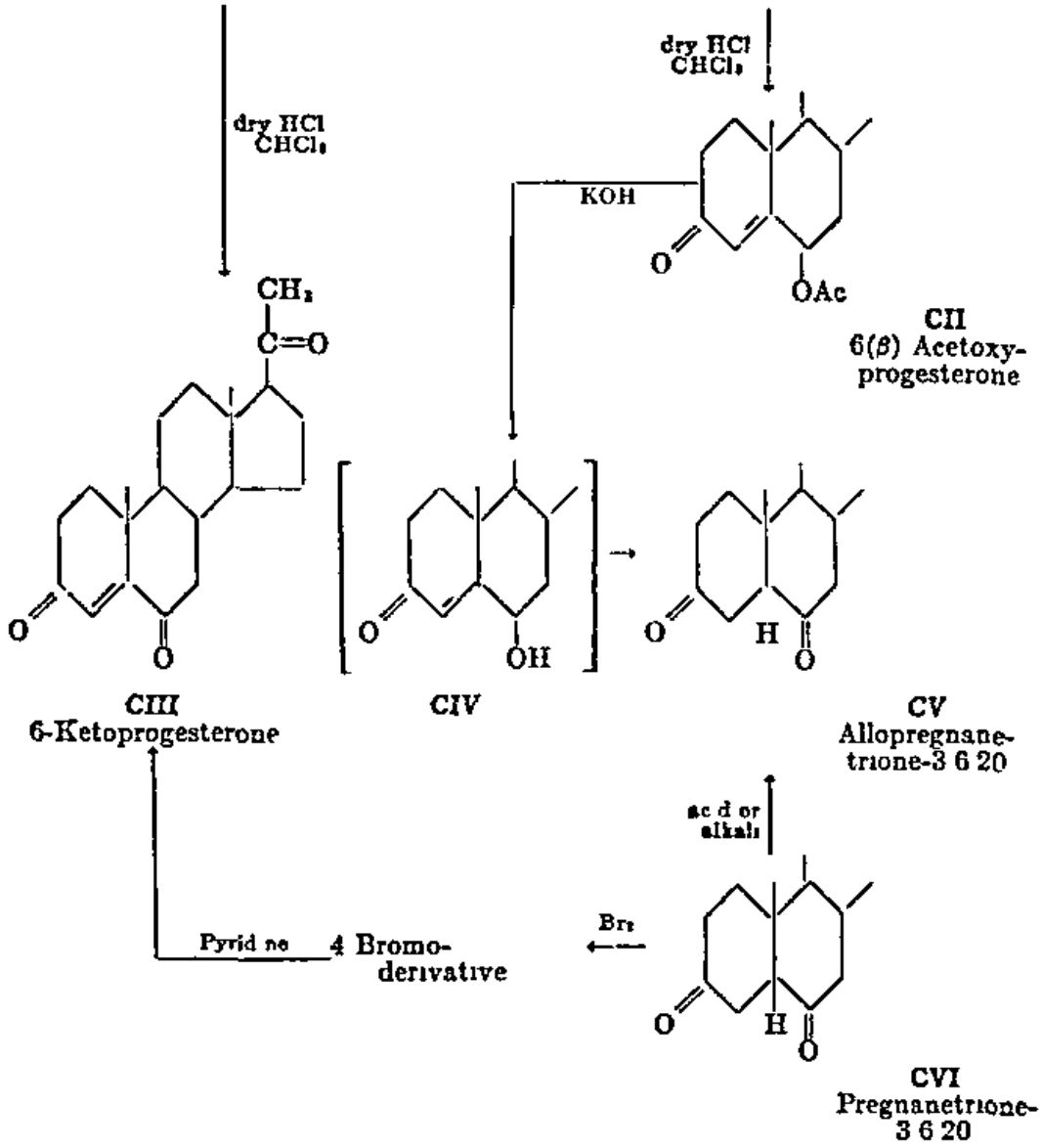
with dry hydrochloric acid in chloroform solution, it undergoes dehydration to yield 6(β)-acetoxyprogesterone (CII), a substance which is active at a 3-mg. dose level in the Corner-Allen test (progesterone is active at a 1-mg. dose level). It was not possible to obtain 6(β)-hydroxyprogesterone from its acetyl derivative, CII; apparently the free compound, CIV, is transformed, during the course of alkaline hydrolysis, to allopregnanetrione-3,6,20 (CV). CV was also obtained from hyodesoxycholic acid by Moffett *et al* (165), who observed that pregnanetrione-3,6,20 (CVI) undergoes rearrangement to CV under the influence of mineral acids or alkali. Reichstein and Fuchs (182) replaced, by indirect methods, the hydroxyl group on carbon 21 in corticosterone

CHART 12

THE PARTIAL SYNTHESIS OF 6 KETO-PROGESTERONE (CIII) AND OF 6(β)-ACETOXY-PROGESTERONE (CII)

(Ehrenstein *et al*.) (Moffett *et al*.)

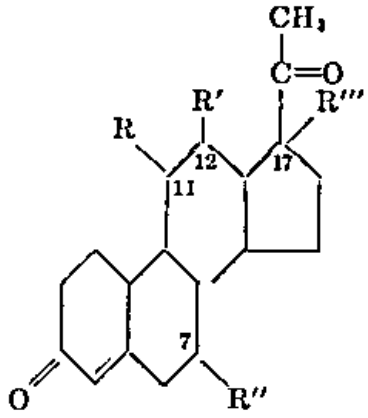




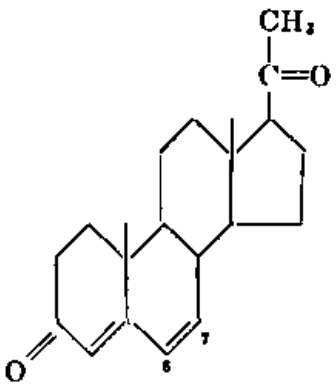
(LXLa) (progestationally inactive in the Clauberg test) to obtain 11-hydroxyprogesterone, CVII, the latter compound possesses little or no progestational activity or, at any rate, less than one sixth the activity of progesterone in the Clauberg test and less than one tenth the activity in the McGinty intrauterine test. These authors (182) also prepared 11-ketoprogesterone (CVIIa) from CVII, CVIIa was not tested for biological activity, however. Hegner and Reichstein (80) subsequently prepared CVIIa from pregnanol-12(α)-dione-3,20, a substance which was derived from desoxycholic acid (XVI). Frhart and co-workers (57) described the preparation of 12(α)-hydroxyprogesterone (CVLIb) from desoxycholic acid (XVI), the substance was found to be lacking in physiological activity. Ehrenstein and Stevens (49) converted pregnanetriol-3(α),7(α),12(α)-one-20 (a substance first prepared by Morsman

et al., 166, from cholic acid) to the diacetate of 7(α),12(α)-dihydroxyprogesterone (CVIIc); the latter preparation was an impure one, however, and no biological tests were reported. The adrenal cortical substance,

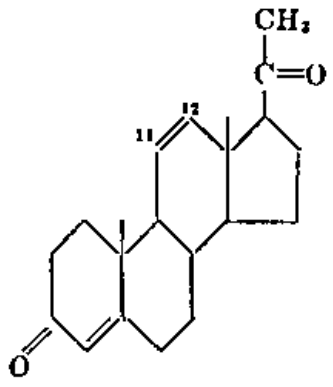
CHART 13



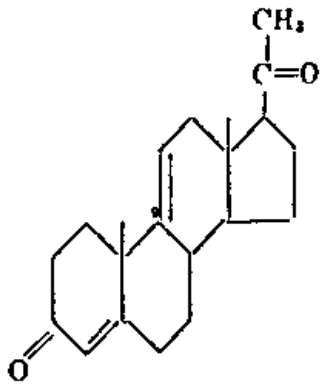
CVII R=OH, R', R'', R'''=H . 11-Hydroxyprogesterone
CVIIa R=O, R', R'', R'''=H : 11-Ketoprogesterone
CVIIb R'=OH, R, R'', R'''=H : 12-Hydroxyprogesterone
CVIIc R', R''=OH, R, R'''=H 7,12-Dihydroxyprogesterone
CVIId R'''=OH, R, R', R''=H 17(β)-Hydroxyprogesterone



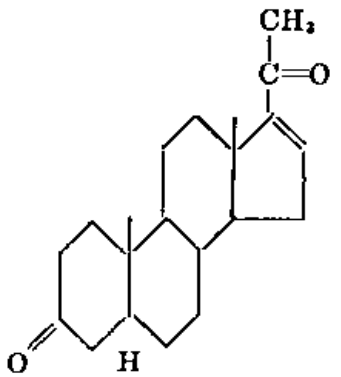
CVIII
6-Dehydroprogesterone



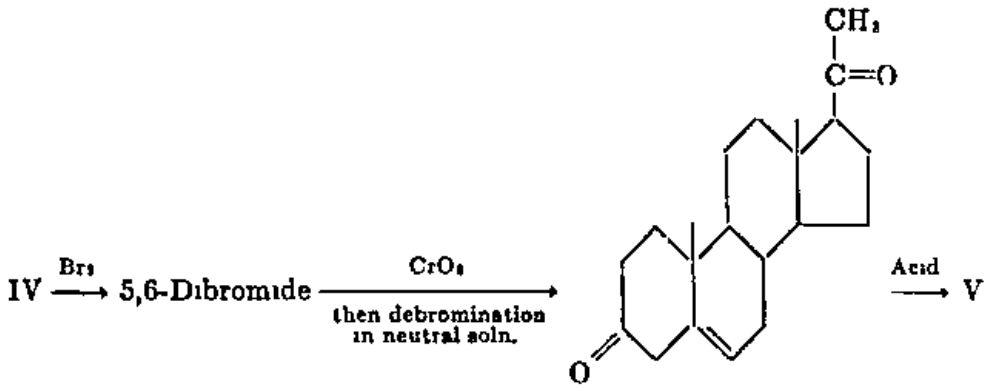
CIX
11-Dehydroprogesterone



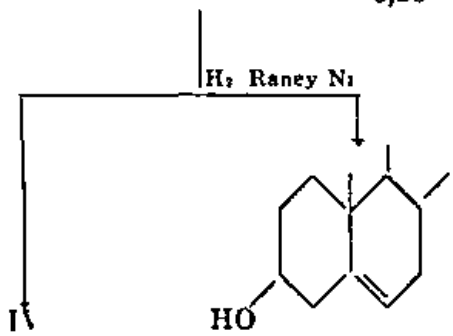
CX
9-Dehydroprogesterone



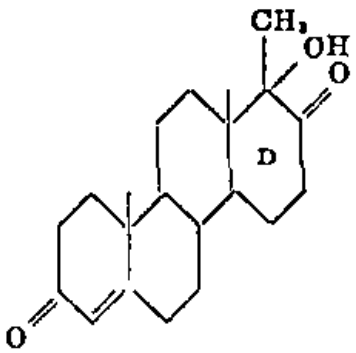
CXI
Δ^{16}-Allopregnenedione-3 20



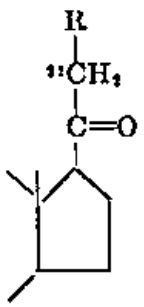
CXII
Δ^{5}-Pregnenedione 3,20



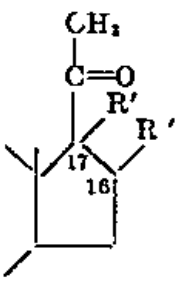
CXIII
Δ Pregnenol 3(α) one-20



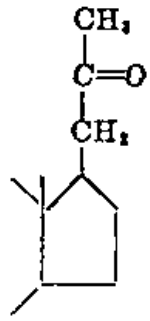
CXIV
Stavely's Progestationally Active Substance



CXV R=CH_3, 21 Methylprogesterone
CXVa R=C_2H_5, 21 Ethylprogesterone



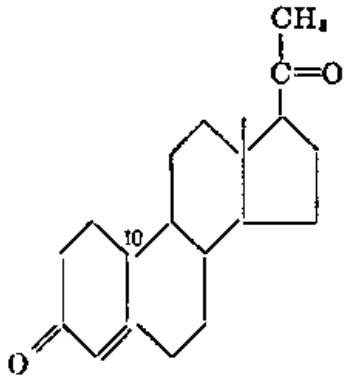
CXVI R′=CH_3, R″=H, 17-Methyl-progesterone
CXVIa R′=H, R″=CH_3, etc, 16-Alkyl progesterone



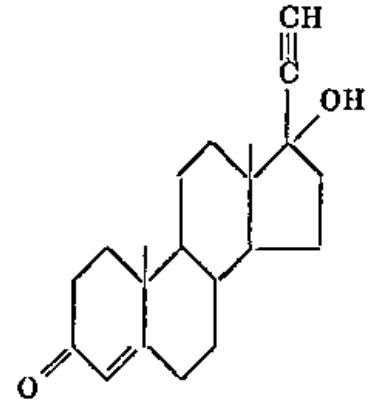
CXVII



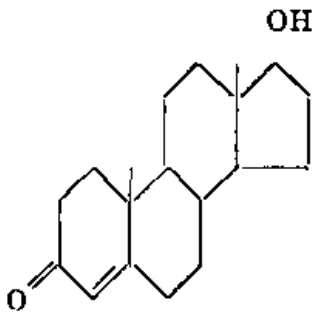
CXVIII
20-Norprogesterone



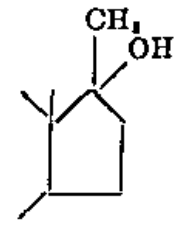
CXIX
10-Norprogesterone



CXX
Pregnenmol-17-one-3
(17 ethinyl Δ^4-androstenol-17-one 3)



CXXI
Testosterone



CXXII
Methyltestosterone

17(β)-hydroxyprogesterone (CVIId) lacks progestational activity (Pfiffner and North, 177) On the other hand, 21-hydroxyprogesterone (or desoxycorticosterone, LXL) has a progestational potency comparable with that of progesterone (V) in the adrenalectomized cat, according to Leathem and Crafts (102), Van Heuverswyn *et al* (216) had previously called attention to the fact that this adrenal cortical compound exerts a progestational effect Kuizenga *et al* (101) report that desoxycorticosterone (LXL) is about one tenth as potent as progesterone (V) in the Corner-Allen test

2 *Unsaturated Derivatives of Progesterone*

Wettstein (226) prepared 6-dehydroprogesterone (CVIII) and found that it possessed approximately one half the biological activity of progesterone (V) CVIII was obtained by oxidizing Δ^5-pregnenol-3(β)-one-20 (IV) by a modified Oppenauer procedure in which quinone was used as the hydrogen acceptor, ordinarily acetone or cyclohexanone is employed as hydrogen acceptor in the reaction which results in the formation of progesterone (V) (see above)

Hegner and Reichstein (79) subjected the benzoyl derivative of CVIIb to thermal degradation and obtained 11-dehydroprogesterone (CIX), it possesses at least one half the biological activity of progesterone in the Clauberg test Shoppee and Reichstein (202) had previously prepared 9-dehydroprogesterone (CX) by hydrochloric acid treatment of CVII, CX was found to be highly active In contrast, the 16-dehydroprogesterone (XLV) first prepared by Butenandt and Schmidt-Thomé (32) is biologically inactive, it was synthesized from dehydroisoandrosterone (III) by the same route as that followed in preparing progesterone (V) (see Section I, 6)

3 *Isomers of Progesterone*

Butenandt *et al* (27) prepared Δ^{16}-allopregnenedione-3,20 (CXI) from androsterone by a route similar to that used in preparing XLV from III (Butenandt and Schmidt-Thomé, 32), CXI is physiologically inactive Butenandt and co-workers (24) had previously described the preparation of Δ^{16}-pregnenedione-3,20 (IX) IX was subsequently obtained by Marker and Rohrmann (136) by oxidative degradation of sarsasapogenin (*VI*)

Butenandt *et al* (26) synthesized Δ^1-allopregnenedione-3,20 (XXXII) and found it to be lacking in progestational activity, XXXII was also prepared by Marker *et al* (148) Westphal and Schmidt-Thomé (65) obtained Δ^5 pregnenedione-3,20 (CXII)[17] on oxidation of the 5,6-dibromide of Δ^5-pregnenol-3(β)-one-20 (IV) followed by dehalogenation in neutral solution This double-bond isomer of progesterone is biologically inactive, it can be readily rearranged to progesterone (V) under the influence of acids The 17-isoprogesterone (XLIX) described by Butenandt *et al* (33) is likewise inactive and it, too, can be readily rearranged to progesterone with acid as a catalyst Neoprogesterone (LVI) was reported by Miescher and Kägi (164) and also by Ruzicka and Meldahl (188) to approach progesterone in its biological activity, but Wettstein (226)

[17] CXII, on partial hydrogenation in alcoholic solution in the presence of Raney nickel yields in addition to Δ^5-pregnenol-3(β)-one-20 (IV) the interesting epimer, Δ^5 pregnenol-3(α)-one-20 (CXIII) (Butenandt and Heusner, 22)

claims that LVI is inactive even at a 30-mg dose level On the other hand, the substance CXIV, which was prepared by Stavely (207) and which like LVI possesses a perhydrochrysene structure, exhibits a slight but distinct progestational action CXIV was obtained by chromatographing Δ^5-pregnenediol-3(β),17(α)-one-20 over alumina (whereupon ring D underwent enlargement to a six-membered ring) and oxidation of the rearrangement product to the corresponding α,β unsaturated ketone

4 *Homologs of Progesterone and Other Progesterone like Compounds*

Wettstein (227) prepared a number of side chain homologs of progesterone (V), some degree of progestational activity is exhibited by 21-methylprogesterone (CXV), but 21-ethylprogesterone (CXVa) and 17-methylprogesterone (CXVI) are biologically inactive [18] Plattner and Schreck (180) described the synthesis of the progesterone homolog CXVII and found it to be biologically inactive Miescher *et al* (86) submitted Δ^4-pregnenediol-20,21-one-3 to periodic acid oxidation and obtained 20 norprogesterone (CXVIII), CXVIII exhibits a slight progestational activity in the rabbit Ehrenstein (46) degraded strophanthidin in order to obtain 10-norprogesterone (CXIX), in about 0 07% yield, as a noncrystalline product which may be a mixture of stereoisomers, CXIX differs from progesterone (V) in that it lacks the angular methyl group between rings A and B (the spatial relationship of these rings in CXIX is not known) It is very interesting that 10-norprogesterone is physiologically as active as progesterone, perhaps even more so

Inhoffen *et al* (92) and also Ruzicka *et al* (187) prepared 17-ethinyl-Δ^4-androstenol-17-one-3 (CXX) (also designated pregneninol-17-one-3, 17-ethinyltestosterone, or more loosely as anhydrohydroxyprogesterone) by Oppenauer oxidation of 17-ethinyl-Δ^5-androstenediol-3,17 (XLIII) CXX possesses a high degree of progestational activity, *e g*, a 2-mg dose of CXX is as effective as 0 6 mg progesterone (V) when injected subcutaneously in the rabbit in the Corner-Allen test Even more astonishing is the observation that a 4-mg dose of CXX is physiologically effective when given to the rabbit by the oral route in view of the fact that progesterone (V) is ineffective by this route These biological findings have been confirmed by other investigators (Emmens and Parkes, 51, Salmon and Salmon, 193, etc) Other products which may be derived from dehydroisoandrosterone (III) and which elicit typical progestational proliferation of the endometrium are testosterone (CXXI), which has a weak progestational action, and 17-methyltestos-

[18] Marker and Crooks (111) have described the preparation of 16-alkylprogesterone homologs (CXVIa), but biological tests were not reported

terone (CXXII), which has about one seventh the biological potency of progesterone (V) (cited by Ruzicka and Rosenberg 191) Selye (200) has listed the relative luteoid (progestational) potency of a number of steroids

5 *Chemical Structure and Biological Activity*

At one time, it was believed that progesterone possessed a high degree of biological specificity, but it appears from the foregoing discussion that this substance may undergo considerable chemical revision and nevertheless retain an appreciable degree of biological activity It is essential, however, that a 3 keto-Δ^4 structure (or its enol acetate) remain It is conceivable that simple nonsteroid substances may yet be synthesized which will possess progestational activity Schueler (197) suggested that those compounds which contain a saturated or partially saturated nucleus of the stilbene type may possess progesterone-like properties Previously, Brownlee, and Duffin (14) reported the preparation of progestationally active compounds which contain the diethylstilbestrol carbon skeleton and have the group —$COCH_3$ in either or both of the benzene rings

II. Metabolism of Progesterone

A Isolation of Progesterone and Related C_{21} Steroids

Progesterone and allopregnanol-3(β)one-20 have been isolated from extracts of corpora lutea and of the adrenal glands (see Table III) It is curious that extracts of hog testes have yielded allopregnanol-3(β) one-20 and Δ^5 pregnenol-3(β) one-20 in addition to androgenic substances (Ruzicka and Prelog, 190) Allopregnanol 3(β) one-20 and a host of other reduction products of progesterone (but not progesterone) have been isolated principally from urinary sources (see Table III) These findings provide much ground for conjecture as to the metabolic relationships of the steroids isolated since many of these compounds represent almost every conceivable stage in the chemical hydrogenation of progesterone

B Some Uncommon C_{21} Steroids of Pregnant Mare Urine

Marker and co-workers (Table IV) have isolated from the urine of pregnant mares a number of highly interesting pregnane derivatives which are difficult to relate to progesterone metabolism, but which may be products of placental origin, these are uranetriol 3(α),11,20, uranediol-3(β),11 and uranol 11 one-3 One of the distinguishing features of these compounds is the presence of an oxygen atom at position 11, a feature characteristic of most of the steroids isolated from the extracts

TABLE III

ISOLATION OF PROGESTERONE AND RELATED C_{21} STEROIDS

Steroid	Formula no	Source	Species	Investigator
Pregnenediones				
Δ^4-Pregnenedione-3,20 (progesterone)	V	Corpus luteum		Butenandt (16)
				Slotta *et al* (203)
			Sow	Allen and Wintersteiner (4), Wintersteiner and Allen (231)
				Hartmann and Wettstein (73)
		Adrenals	Ox	Beall (9)
				Beall and Reichstein (10)
Pregnanediones				
Pregnanedione-3,20	XXVIII	Pregnancy urine	Mare	Marker *et al* (132)
Allopregnanedione-3,-20	XXX			
Pregnenolones				
Δ^5-Pregnenol-3(β)-one-20	IV	Testis	Hog	Ruzicka and Prelog (190)
Pregnanolones				
Pregnanol-3(α)one-20	LVIII	Pregnancy urine	Man	Marker and Kamm (120)
				Pearlman and Pincus (173)
			Sow	Marker and Rohrmann (135)
Allopregnanol-3(α)-one-20	CXXXI	Pregnancy urine	Man	Marker *et al* (124)
Allopregnanol-3(β) one 20	LXII	Corpus luteum	Sow	Butenandt, and Westphal (35)
				Slotta *et al* (203)
			Sow	Wintersteiner and Allen (231)
			Sow	Hartmann and Wettstein (73)
		Adrenals	Beef	Beall (9), Beall and Reichstein (10)
		Pregnancy urine	Man	Pearlman *et al* (175)
			Mare	Marker *et al* (132)
				Heard and McKay (78)
			Sow	Marker and Rohrmann (135)
		Testis	Hog	Ruzicka and Prelog (190)

TABLE III (*Continued*)

Steroid	Formula no	Source	Species	Investigator
Pregnenediols				
Δ^5-Pregnenediol 3(β), 20(α)	LXLIV	Pregnancy urine	Mare	Marker and Rohrmann (133)
		Urine adrenal tumor	A boy	Hirschmann and Hirschmann (84)
			A girl	Schiller *et al* (195)
Pregnanediols				
Pregnanediol-3(α), 20(α)	XI	Pregnancy urine	Man	Marrian (151)
				Butenandt (15)
			Chimpanzee	Fish *et al* (61)
			Cow	Marker (107)
			Mare	Marker *et al* (121)
		Nonpregnancy urine	Man	Venning and Browne (219)
				Marker *et al* (140)
		Nonpregnancy urine (ovariectomy)	Man	Hirschmann (81)
		Adult male urine	Man	Engel *et al* (52)
			Bull	Marker *et al* (149)
		Urine* adrenal tumor and hyperplasia	Man	Mason and Kepler (155) (and others)
Pregnanediol 3(β), 20(α)[b]	CXXIII	Urine adrenal tumor and hyperplasia	Man	Mason and Kepler (155)
Allopregnanediol 3(α), 20(α)	LXIV	Pregnancy urine	Man	Hartmann and Locher (72)
			Cow	Marker (107)
			Mare	Marker *et al* (121)
		Nonpregnancy urine	Man	Marker *et al* (149)
		Adult male urine	Bull	
Allopregnanediol-3(β),20(α)	LXV	Pregnancy urine	Man	Marker and Rohrmann, (134)
			Cow	Marker (107)
			Mare	Marker and Rohrmann (134)
		Adult male urine	Bull	Marker *et al* (149)
Allopregnanediol 3(β),20(β)	LXIII	Bile	Ox	Pearlman (172)
Pregnanols				
Pregnanol-3(α)	CXXIV	Pregnancy urine	Man	Marker and Lawson (130)

* The urinary content of pregnanediol-3(α),20(α) in cases of adrenal hyperplasia and tumor is considerably above normal values [b] Tentative identification

of the adrenal cortex The urane compounds are peculiar in that the hydrogen atom on carbon 9 is considered to have a spatial configuration opposite to that of the naturally occurring steroids There is indirect evidence, which is by no means conclusive, that the configuration of rings A and B in the urane products corresponds to that in *cis*-decalin, *i e*, the urane compounds may be 9-isopregnane derivatives

Marrian, Marker, and respective co-workers isolated from pregnant mare urine allopregnanetriol-3(α),16,20 (pregnanetriol B) (Table IV)

TABLE IV

SOME UNCOMMON C_{21} STEROIDS ISOLATED FROM PREGNANT MARE URINE

Steroid	Formula no	Investigator
Allopregnanetriol 3(α),16,20 (pregnanetriol B)	CXXV	Smith *et al* (204) Haslewood *et al* (75) Marker *et al* (122)
Uranetriol 3(α),11,20[a]	CXXVI	Marker *et al* (122,125)
Uranediol 3(β),11	CXXVII	Marker *et al* (141)
Uranol 11-one-3	CXXVIII	Marker *et al* (132)
Compound Z, Δ^{16} allopregnenol-3(β)-one-20 (identification tentative)	CXXIX	Klyne and Marrian (98)
Compound Y (possibly identical with uranediol-3(β),11)		Klyne, (97)

[a] This compound probably occurs in human pregnancy urine since high vacuum distillation of the oxidized neutral ketonic fraction yielded uranetrione (Marker, 60)

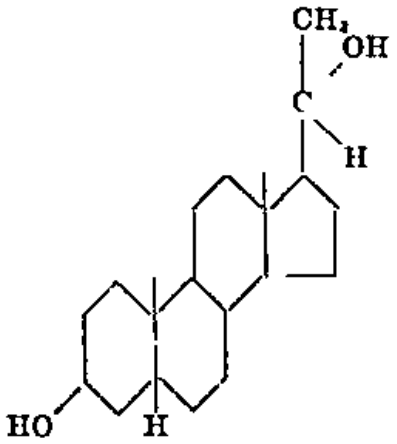
The structural formula first assigned to this substance has undergone considerable revision at the hands of the investigators who first isolated this substance (Smith *et al*, 204, Haslewood *et al*, 75, Odell and Marrian, 170, and Marker *et al*, 128) The structure finally assigned to pregnanetriol B by Marker *et al* (147,144) is indicated by CXXV (Chart 14) Quite recently Klyne and Marrian (98) isolated from pregnant mare urine compound Z, a substance which may be identical with Δ^{16}-allopregnenol-3(β)-one-3 Compound Y was also isolated from the same source by Klyne (97), it may possibly be identical with the uranediol 3(β),11 obtained by Marker *et al* (141)

C Detection of Progestationally Active Material in Body Tissues and Fluids

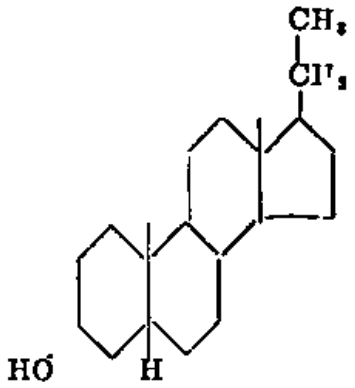
It is an astonishing fact that whereas the organism may excrete, relatively speaking, prodigious amounts of pregnane derivatives, the presence of progesterone or of progestationally active material can hardly be detected in the blood or urine Even the demonstration of progestin

activity in extracts of the corpus luteum[19] and of the placenta is not attended without difficulty and often as not negative results are obtained

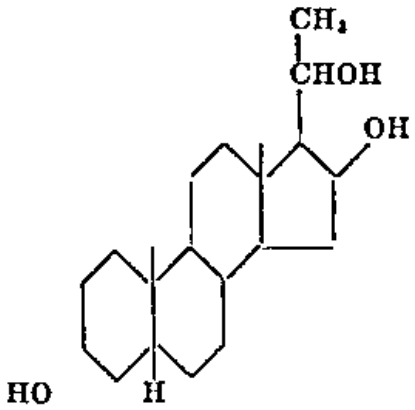
CHART 14



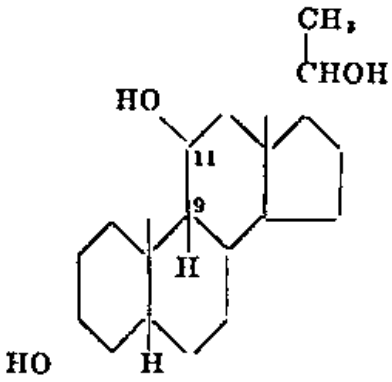
CXXIII
Pregnaned ol-3(β) 20(α)



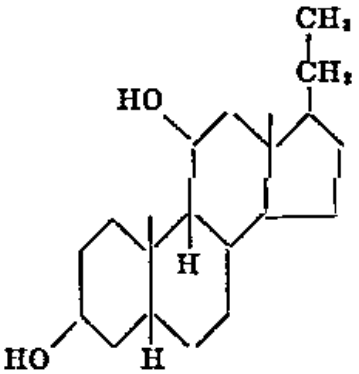
CXXIV
Pregnanol 3(α)



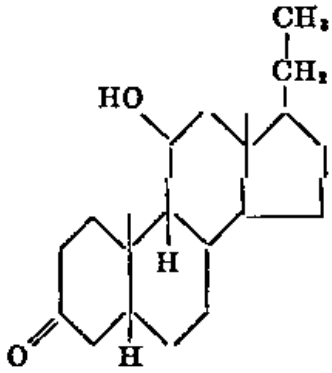
CXXV
Pregnanetriol B or
allopregnanetriol 3(α) 16 20



CXXVI
Pregnanetriol A or
uranetriol 3(α) 11 20

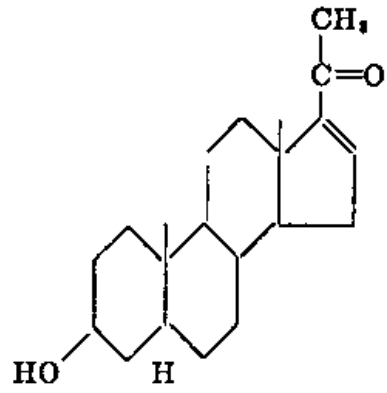


CXXVII
Uraned ol 3(β) 11

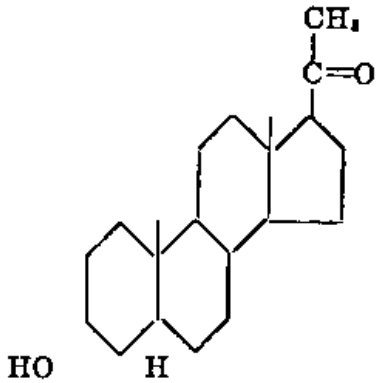


CXXVIII
Uranol 11-one-3

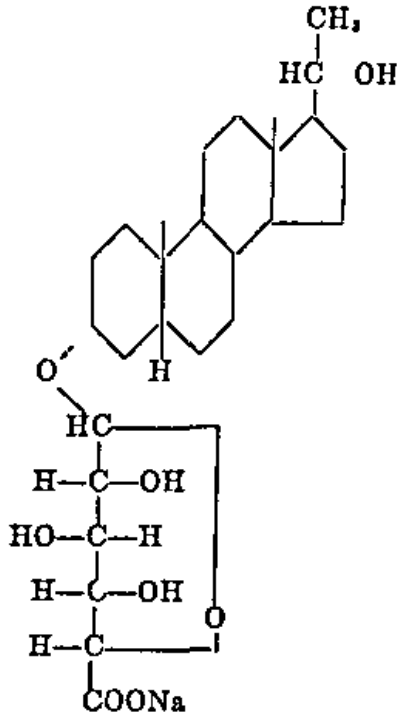
[19] One kg of corpus luteum tissue of the sow contains approximately 30–50 rabbit units (Allen 1)



CXXIX
Δ^{16} Allopregnenol 3(β)-one 20



CXXXI
Allopregnanol 3(α)-one-20



CXXX
Sodium pregnanediol 3(α) 20(α) glucuronidate

The high requirement and the small quantity of progestin found in body tissues suggest continuous production and almost immediate utilization of progesterone (12)

Loewe and Voss (103) demonstrated the presence of only one Corner Allen unit in 20 l human pregnancy urine, one may expect to find as much as 1 g pregnanediol 3(α),20(α) in an equivalent volume of late pregnancy urine Erhardt and Hagena (56) found no progestin in the urine of two women receiving 30 and 50 Clauberg units of progesterone by injection Hamblen *et al* (69) made similar observations Clauberg *et al* (41) failed to demonstrate progestin in 335 ml human blood (menstrual or circulating) Bloch (12) found less than 1 Corner Allen rabbit unit of progestin in 8 and 12 l sow blood (but neither the stage of the oestrus cycle nor the possibility of pregnancy in these animals was known) Bloch also found that the total circulating blood of a pregnant rabbit contains less than 1 rabbit unit of progestin, the progestin assay of the circulating blood of pregnant women gave negative results (as much as 500 ml blood was tested) Haskins (74), employing the sensitive intrauterine technique devised by McGinty *et al*, detected the

presence of progestationally active material in the serum of pregnant women in amounts equivalent to about 0 13 μg progesterone/ml serum De Allende (43) found the progesterone content/ml blood of a *Macacus rhesus* monkey to vary between a maximum of 0 25 to 2 5 μg and a minimum of 0 06 to 0 12 μg during a complete menstrual cycle

D Probable Sites of Progesterone Elaboration Corpus Luteum, Placenta, and Adrenal Cortex

Prima facie evidence for the elaboration of progesterone in the corpus luteum and in the adrenal cortex is the actual isolation of progesterone from extracts of these organs (see Table III) Progesterone has not as yet been isolated from extracts of the placenta but there is reason (*vide infra*) to believe that this organ is responsible for the elaboration of progestationally active material Mazer and Goldstein (157) detected progestin in the placenta of a six month pregnant woman The presence of progestin in early as well as full term placentas has been demonstrated by some investigators, but others have often obtained negative results [10] Smith and Kennard (205) detected progestin in full term placentas in several instances Apparently the quantity of progestationally active material in extracts of the human placenta is very small, there seems to be an absence of progestin in the placenta of slaughtered cattle (Mazer and Goldstein, 157) It cannot be assumed that the progestationally active material of placental extracts is due to progesterone because the progesterone molecule does not possess the high degree of biological activity as was at one time believed (see Section I, C, 5) It would be very desirable therefore to isolate and identify the progestin principle of placental extracts With regard to the progestational activity exhibited by extracts of the adrenal cortex, progesterone and desoxycorticosterone are contributing factors since both substances have been isolated from this source, desoxycorticosterone is weaker, however, than progesterone in its biological (progestin) potency (see Section I, C, 5)

E Relative Importance of the Endocrine Organs in Progesterone Elaboration

Although progesterone is not the sole metabolic source of pregnanediol-3(α),20(α) (see Section II, F), the rate of excretion of the latter substance may serve as an excellent index of the rate of progesterone elaboration and metabolism especially if such studies are correlated with the formation, changes in histological picture, etc of certain glandular

[10] For citation of literature references see Bloch (12) Failure to detect progestin in tissue extracts may be due to the incomplete removal of estrogens from these extracts the estrogens inhibit progestin in its biological effects (Allen and Meyer, 2)

organs, *e g*, the corpus luteum Thus it appears that in women with normal menstrual cycles, the corpus luteum is the major source of progesterone [21] During pregnancy, the placenta appears to be major source of progesterone especially in the later stages of gestation The adrenal cortex probably contributes to a small extent to the pool of pregnanediol-3(α),20(α) since desoxycorticosterone as well as progesterone serve as metabolic sources of pregnanediol (Table V) In cases of adrenal cortical hyperplasia (Salmon *et al*, 192, Anderson *et al*, 5, Hirschmann, 83, Mason and Kepler, 155) and of adrenal cortical tumor (Butler and Marrian, 38, Mason and Kepler, 155), the urinary excretion of pregnanediol-3(α),20(α) (as well as that of other steroids) may be considerably elevated above normal

TABLE V

ESTABLISHED METABOLIC PRECURSORS OF PREGNANEDIOL-3(α),20(α)

Metabolic precursor	Experimental subject	Investigator
Progesterone (V)	Men	Buxton and Westphal (40) Hamblen *et al* (71)
	Women	Venning and Browne (219)
	A hysterectomized woman	Muller (167)
	Hysterectomized patients	Buxton (39) Venning and Browne (220) Jones and Te Linde (93)
	Rabbits	Heard *et al* (76)
	(Intact and ovariectomized hysterectomized)	Hoffman and Browne (88)
	(Intact and castrated male)	Hoffman (87)
Desoxycorticosterone (LXL)	A man	Cuyler *et al* (42)
	Rabbit	Westphal (223) Hoffman *et al* (89)
	Ovariectomized chimpanzee	Fish *et al* (62)
	Ovariectomized chimpanzee, male patients (hypogonadal), male and female patients (Addison's disease)	Horwitt *et al* (90)
Cholesterol (I)	A pregnant woman	Bloch (11)
Δ^5 Pregnenol 3(β)one-20 (IV)	Men, a postmenopausal woman, rabbits	Pearlman and Pincus (174)

[21] For a more extensive treatment of this and closely related subjects, see review articles by Hoffman (87) and by Pincus and Pearlman (176)

The precise role of the testis in pregnanediol formation remains to be determined In sharp contrast to the adult males of most species, the bull excretes relatively enormous quantities of pregnanediol-3(α),20(α) (Marker, 108), the urine of steers is lacking in this steroid Most pertinent to these observations is the recent finding that Δ^5 pregnenol 3(β)-one-20, a possible metabolic precursor of progesterone, occurs in extracts of hog testis (Table III, page 448) It is conceivable that the testis elaborates pregnenolone and that its conversion to progesterone occurs in the adrenal cortex

F Established Pathways in the Metabolism of Progesterone and Related C_{21} Steroids

Progesterone, desoxycorticosterone, and Δ^5-pregnenol-3(β)-one-20 (all of which occur in the organism, see Table III) are definitely known to undergo metabolic transformation into pregnanediol 3(α),20(α) No product other than pregnanediol-3(α),20(α) has been isolated in metabolism studies[22] (Table V) in which the three above-mentioned steroids were administered in massive doses, not even after the oral administration of as much as 5 g progesterone to two women (Venning, 217) This is most surprising in view of the fact that pregnanolones and stereoisomers of pregnanediol (Table III) are excreted during pregnancy, a period during which the rate of progesterone elaboration is generally believed to be at a very high level

Of great fundamental importance is the recent finding by *Bloch* (11) that cholesterol can serve as a metabolic source of pregnanediol-3(α),-20(α) This investigator administered deuterium-containing cholesterol to a woman in the eighth month of pregnancy, pregnanediol-3(α),20(α) was isolated from the urine as the sodium glucuronidate On the basis of the isotope concentration of the urinary pregnanediol and of the blood cholesterol, it was estimated that one half to two thirds of the pregnanediol excreted arose by the degradation of cholesterol It was suggested that the direct conversion of cholesterol to progesterone is a normal process

As an aid to apprehending the chemical mechanisms involved in the metabolism of progesterone in mammals, experiments carried out with

[22] It may be of some interest to mention that pregneninolone (CXX) (anhydrohydroxyprogesterone), which is biologically active when administered orally, is not converted to pregnanediol glucuronide (Hamblen *et al*, 70) Previously Allen *et al.* (3) reported the excretion of sodium pregnanediol glucuronidate in the urine following the oral administration of anhydrohydroxyprogesterone (CXX) to patients these investigators conceded, however, that the product they had isolated required chemical investigation.

bacteria and yeast may be of considerable value Mamoli *et al* (106) found that the reduction of progesterone to pregnanedione-3 20[23] could be effected by a *Bacillus putrefactus* (yeast water or brei) Mamoli (105) observed that Δ^5 pregnenol 3(β) one 20 was transformed on incubation with yeast (bacteria?) to progesterone This transformation is of special interest in that it involves a shift of the 5 6 double bond to the 4 5 position simultaneous with the oxidation of the 3 hydroxyl group (*cf* Oppenauer oxidation) Similar oxidation of 3(β) hydroxy Δ^5 steroids other than those belonging to the C_{21} series can be effected by various microorganisms[24] in some instances pure cultures of bacteria have been utilized *e g* species of *Proactinomyces* (Turfitt 213)

G Conjugation of Pregnanediol and Related C_{21} Steroids

The structure of the pregnanediol glucuronide found in human pregnancy urine (Odell and Marrian 169) (Venning and Browne 218) has been completely established in recent years Heard *et al* (77) acetylated the sodium salt of pregnanediol glucuronide and obtained a crystalline nonacidic acetate apparently a triacetate lactone Mild acid hydrolysis of the latter product yielded a relatively large amount of pregnanediol 3(α) 20(α) 20-monoacetate but none of the 3 monoacetate *It was* deduced that conjugation must exist between the 3 hydroxyl group (and not the 20 hydroxyl group) of pregnanediol and the glucosidic hydroxyl group of the uronic acid Very shortly afterward Huebner *et al* (91) achieved the partial synthesis of the 3 β-*d* glucuronide of pregnanediol 3(α) 20(α) and established its identity with the urinary product Thus it is positively established that the carbohydrate component of the pregnanediol complex is *d* glucuronic acid and that the configuration of the glucosidic linkage is β The structural formula of sodium pregnanediol glucuronidate is indicated by CXXX

Pregnanediol (3) 20(α) is not the only C_{21} steroid excreted as a uronic acid complex Marrian and Gough (153) have shown that sodium pregnanediol glucuronidate prepared by current procedures from human pregnancy urine contains about 20% of a water soluble derivative of pregnanol 3(α) one-20 as an impurity This substance believed to be sodium pregnanol 3(α) one 20 glucuronidate has been isolated in a somewhat impure state (Sutherland and Marrian 211) with the aid of Girard's reagent T Mason and Kepler (155) have recently adduced evidence

[23] The excretion of pregnanediol in the feces of rabbits following the oral administration of progesterone (Hoffman and Browne 88) might result from the action of bacteria in the gastrointestinal tract pregnanedione 3 20 might be formed as an intermediate

[24] For a recent review of phytochemical transformations of the steroids see Pincus and Pearlman (176)

that the pregnanetriol-3(α),17,20, first isolated by Butler and Marrian (38) from the urine of women with adrenal hyperplasia, occurs as the glucuronide

Marrian (152) has pointed out that the widespread occurrence in animal tissues of "β glucuronidase," an enzyme specific for the hydrolysis of β glucuronides, is of considerable interest in view of the important role of glucuronic acid conjugation in "detoxication" and in steroid metabolism Fishman (63) has furnished evidence for the role of β glucuronidase as a catalyst in the synthesis of glucuronides, he proposes that formation of glucuronides of pregnanediol (and estriol) be regarded as one of the processes of "metabolic conjugation" rather than one exclusively of the process of "detoxication " The site of conjugation of pregnanediol with glucuronic acid is not known but it is generally believed to be the liver

Certain of the C_{21} steroids excreted in pregnancy urine may exist also as sulfuric acid esters Klyne (97) and Klyne and Marrian (98) reported the isolation, from the urine of pregnant mares, of the sulfuric acid esters of compounds Y and Z (see Table IV for the possible structure of these C_{21} compounds)

Although pregnanediol-3(α),20(α) is found in human urines and in the urine of the chimpanzee, cow, bull, and mare (Table III, page 448), pregnanediol glucuronide has been isolated only from human urine and from the urine of rabbits (after progesterone administration) (Hoffman, 87) The following attempts to isolate the complex from the urine of other species have been unsuccessful[25] (*a*) Westphal and Buxton (224), from normal and pregnant rabbits, normal and pregnant cats and from monkeys (after progesterone administration), (*b*) Fish *et al* (61) (unpublished observations), from guinea pigs (after administration of progesterone and of sodium pregnanediol glucuronidate), (*c*) Elder (50) from pregnant chimpanzee, (*d*) Strickler *et al* (210), from the bull

H Site of Progesterone Catabolism

On the basis of studies carried out on normal men and women, hysterectomized women, intact and ovariectomized-hysterectomized rabbits, and intact and castrated male rabbits (Table V, page 454), it appears that the uterus, corpus luteum, and testis are not essential for the conversion of progesterone to pregnanediol-3(α),20(α) This statement should not be interpreted to mean that these organs do not participate in progesterone metabolism, but rather that the site of pregnanediol

[25] The isolation of the pregnanediol complex from animal sources appears to be attended with difficulty since first attempts by Heard *et al* (76) to isolate the complex from rabbit urine were unsuccessful due to the presence of impurities

formation is not confined to these organs It is not known whether or not the transformation of progesterone to pregnanediol can occur in the adrenalectomized animal and so it is not possible to evaluate at the present time the role of the adrenal cortex in this regard There is, on the other hand, indirect evidence that the metabolic conversion of progesterone to pregnanediol or to some other biologically inactive substance may occur in nonendocrine organs of the body, particularly in the liver For example, Engel (54) injected progesterone into infantile rabbits (in the Clauberg test) by the subcutaneous route and by injection into the portal vein, the progestational activity of progesterone applied intraportally was inferior in low amounts (0 5 or 1 mg) but doses above 2 mg were fully active Mussio-Fournier *et al* (168) found that progesterone injected into the liver or spleen of rabbits was less effective in inducing progestational changes than when injected subcutaneously, Fels and Monaco (58) were not able to confirm these results, however, Kochakian *et al* (99) carried out experiments which indicate that the liver may be an important site for the metabolism of progesterone These investigators implanted pellets of progesterone subcutaneously, in the muscle, in a fold of the mesentery (drainage to the liver), and in the kidney of female rabbits Although as much as 20 mg of progesterone was absorbed from the pellet implanted in the mesentery, no endometrial reaction was observed, only 2 6 mg progesterone was absorbed from the pellet implanted in the muscle, yet a strongly positive endometrial response was observed The kidney as well as the liver may be involved in the biological inactivation of progesterone but the kidney to a much lesser extent Selye *et al* (199) observed that the anesthetic action of orally administered progesterone is greatly enhanced by partial hepatectomy The recent work of Masson and Hoffman (156) also points strongly to the liver as a major site for the biological inactivation of progesterone These investigators found that as much as 200 mg progesterone had to be given by gavage to adult rabbits to obtain an endometrial proliferation comparable to that obtained in the partially-hepatectomized animal with 25 mg Destruction of progesterone in, or its poor absorption from, the gastrointestinal tract does not appear to be a likely explanation[25] of the ineffectiveness of the orally administered progesterone since as much pregnanediol appeared in the urine of rabbits following the oral administration of progesterone as when the same dose was given subcutaneously (Hoffman, 1944, unpublished observations)

[25] This factor cannot be entirely disregarded in view of the report by Hoffman and Browne (88) that only those rabbits receiving progesterone orally excrete pregnanediol in the feces the extent of conversion is about 7 %

It is assumed that the progesterone absorbed from the gastrointestinal tract must pass directly through the liver before reaching the uterus

As further indirect evidence for the role of the liver in the metabolism of progesterone (or of closely related C_{21} steroids), one may cite the observation by Pearlman and Pincus (174) that pregnanediol-3(α),20(α) is excreted in the bile of a postmenopausal woman following the oral administration of Δ^5-pregnenol-3(β) one-20, a hypothetical metabolic precursor of progesterone Pertinent also is the recent isolation of allo-pregnanediol-3(β),20(β) from normal ox bile (Pearlman, 172)

The above-cited evidence notwithstanding, it remains to be proved that the chief site of progesterone metabolism is indeed in the liver According to the observations of Zondek (233) (confirmed by Engel, 53) the liver does not inactivate progesterone *in vitro*

I Some Unsolved Problems in Progesterone Metabolism

From the foregoing presentation, it is apparent that the metabolism of progesterone is intimately linked with the metabolism of cholesterol[27] and with that of other steroid hormones It is perhaps equally apparent that there are many gaps in our knowledge of this subject Thus there remain to be determined the various metabolic routes which are followed in the course of formation of pregnanediol from the steroids listed in Table V For example, with respect to the *in vivo* conversion of choles terol to pregnanediol, it is a matter of conjecture as to whether the side chain of cholesterol is degraded to yield pregnenol-3(β) one-20 and the latter substance converted to pregnanediol via progesterone What are the metabolic routes involved in the conversion of progesterone to pregnanediol? Is progesterone first converted to pregnanedione-3,20, then to pregnanol 3(α) one-20, and finally to pregnanediol? What of the numerous C_{21} steroids listed in Tables III and IV? How are these substances to be related to the metabolism of progesterone? Venning (217) posed the interesting question of whether progesterone gives rise to both pregnanediol and pregnanolone during pregnancy, or whether the pregnanolones are derived from the metabolism of the adrenal substances

Is progesterone converted to any extent to 17 ketosteroids? This question has been discussed previously (Pincus and Pearlman 176), there does not appear to be convincing evidence that such a transforma-tion actually occurs It might be pertinent to mention that Longwell and Gassner (104) have recently reported that the amount of androgenic material excreted in the feces of the cow is considerably increased during

[27] The recent preparation of radioactive cholestenone containing C^{14} in ring A (Turner 214) may be a factor of considerable importance in advancing our knowledge regarding the metabolism of cholesterol and of progesterone

pregnancy The androgenic principle has not as yet been isolated The formation of androgens during pregnancy may be a process independent of that involved in the elaboration of progesterone

Venning (217) noticed that there is a high excretion of corticoid substances in the urine during human pregnancy, an increased activity of the adrenal glands was postulated It is significant that Marker (Table III) had previously demonstrated the excretion of 11-oxygenated steroids in the urine of pregnant women and of pregnant cows Are these substances formed in the placenta or in the adrenal cortex? One also wonders whether progesterone may not be an immediate metabolic precursor of desoxycorticosterone and of other adrenal cortical steroids in view of their close structural resemblance

REFERENCES

1 Allen, W M *J Biol Chem* **98**, 591 (1932)
2 Allen W M , and Meyer, R K *Am J Physiol* **106**, 55 (1933)
3 Allen, W M , Viergiver, E , and Soule, S D *J Clin Endocrinol* **4**, 202 (1944)
4 Allen W M , and Wintersteiner, O *Science* **80**, 190 (1934)
5 Anderson, A F , Hain, A M , and Patterson J *J Path Bact* **55**, 341 (1943)
6 Aston J G , and Greenburg, R B *J Am Chem Soc* **62**, 2590 (1940)
7 Baeyer, A V and Villiger, V *Ber* **32**, 3625 (1899)
8 Barbier P , and Roquin, R *Compt rend* **156**, 1443 (1913), Wieland, H , Schlichtling, O , and Jacobi, R. *Z physiol Chem* **161**, 80 (1926)
9 Beall D *Biochem J* **32**, 1957 (1938)
10 Beall, D and Reichstein, T *Nature* **142**, 479 (1938)
11 Bloch, K *J Biol Chem* **157**, 661 (1945)
12 Bloch P W *Endocrinology* **20**, 307 (1936)
13 Bretschneider, H *Ber* **74**, 1360 (1941)
14 Brownlee G , and Duffin, W M Brit Patent 550,262 (1941, 1942)
15 Butenandt, A *Ber* **63**, 659 (1930)
16 Butenandt A *Wien klin Wochschr* **47**, 936 (1934)
17 Butenandt A , and Cobler, H *Z physiol Chem* **234**, 218 (1935)
18 Butenandt A , Cobler, H , and Schmidt, J *Ber* **69**, 448 (1936)
19 Butenandt A , Dannenbaum, H , Hanisch, G , and Kudszus, H *Z physiol Chem* **237**, 57 (1935)
20 Butenandt, A , and Fleischer, G *Ber* **68**, 2094 (1935)
21 Butenandt A , and Fleischer, G *ibid* **70**, 96 (1937)
22 Butenandt A , and Heusner, A *ibid* **72**, 1119 (1939)
23 Butenandt A , and Mamoli L *ibid* **68**, 1847 (1935)
24 Butenandt A , and Mamoli, L *ibid* **68**, 1853 (1935), Masch, L W , Dissertation Danzig, 1938
25 Butenandt A and Mamoli, L *ibid* **68**, 1854 (1935), Butenandt, A , and Wolff A *ibid* **68**, 2091 (1935)
26 Butenandt A , Mamoli, L , Dannenberg, H , Masch, L W , and Paland, J *ibid* **72**, 1617 (1939)
27 Butenandt A , Mamoli, L , and Heusner, A *ibid* **72**, 1614 (1939)
28 Butenandt, A , and Muller, G *ibid* **71**, 191 (1938)
29 Butenandt A , and Ruhenstroth Bauer G *ibid* **77**, 397 (1944)
30 Butenandt, A , and Schmidt, J *ibid* **67**, 1893 (1934)

31 Butenandt, A , and Schmidt, J *ibid* **67**, 1901 (1934)
32 Butenandt, A , and Schmidt-Thomé, J *ibid* **71**, 1487 (1938), *ibid* **72**, 182 (1939)
33 Butenandt, A , Schmidt-Thomé, J , and Paul H *ibid* **72**, 112 (1939)
34 Butenandt, A., Tschernıng K , and Hanisch, G *ibid* **68**, 2097 (1935)
35 Butenandt, A , and Westphal, U *ibid* **67**, 1140 (1934)
36 Butenandt, A , Westphal, U , and Cobler, H *ibid* **67**, 1611 (1934), Butenandt, A , and Schmidt J , *ibid* **67**, 1901 (1934) Butenandt A , and Westphal, U , *ibid* **67**, 2085 (1934)
37 Butenandt, A , and Wolff, A *ibid* **72**, 1121 (1939)
38 Butler, G C , and Marrian, G F *J Biol Chem* **119**, 565 (1937) Butler G C , and Marrian, G F *ibid* **124**, 237 (1938), Marrian, G F , and Butler, G C , *Nature* **142**, 400 (1938)
39 Buxton, C L *Am J Obstet. Gynecol* **40**, 202 (1940)
40 Buxton C L , and Westphal, U *Proc Soc Exptl Biol Med* **41**, 284 (1939)
41 Clauberg C H , Thiel, H W , and Ziecker, R. *Arch Gynäkol* **152**, 61 (1933)
42 Cuyler, W K., Ashley C , and Hamblen, E C *Endocrinology* **27**, 177 (1940)
43. de Allende, I L C *Proc Soc Exptl Biol Med* **44**, 534 (1940)
44 Dirscherl, W , and Hanusch, F *Z physiol Chem.* **252**, 49 (1938)
45 Ehrenstein M *J Org Chem* **4**, 506 (1939)
46 Ehrenstein, M *ibid* **9**, 435 (1944), Allen, W M , and Ehrenstein, M , *Science* **100**, 251 (1944)
47 Ehrenstein, M , and Decker, M T *J Org Chem* **5**, 544 (1940)
48 Ehrenstein, M , and Stevens, T O *ibid* **5**, 318 (1940), **6**, 908 (1941)
49 Ehrenstein, M , and Stevens, T O *ibid* **5**, 660 (1940)
50 Elder, J H *Proc Soc Exptl Biol Med* **46**, 57 (1941)
51 Emmens, C W , and Parkes, A S *J Endocrinol* **1**, 332 (1939)
52 Engel, P *Endocrinology* **35**, 70 (1944)
53 Engel, P *ibid* **38**, 215 (1946)
54 Engel, L L ,Thorn, G W , and Lewis R. A. *J Biol Chem* **137**, 205 (1941)
55 Ercoli, A , and Mamoli, L *Chimica e industria* 435 (1937), cited by Miescher, K., and Kägi, H *Helv Chim Acta* **22**, 184 (1939)
56 Erhardt, K , and Hagena A *Endokrinologie* **16**, 51 (1935)
57 Ehrhart, G , Ruschig W , and Aumüller, W *Angew Chem* **52**, 363 (1939), Bockmühl, M , Ehrhart, G , Ruschig, W , and Aumüller W U S Patent 2 142,170, *Chem Abstracts* **33**, 3078 (1939), *Chem Centr* **1939, II**, 170
58 Fels, E , and Monaco H *Rev soc argentina biol* **17**, 385 (1941)
59 Fernholz, E *Ann* **507**, 128 (1933)
60 Fernholz, E *Ber* **67**, 1855, 2027 (1934)
61 Fish, W R., Dorfman, R I , and Young W C *J Biol Chem* **143**, 715 (1942)
62 Fish, W R , Horwitt, B N , and Dorman, R I *Science* **87**, 227 (1943)
63 Fishman, W H *Federation Proc* **6**, 251 (1947), *J Biol Chem* **127**, 367 (1939); **131**, 225 (1939), **136**, 229 (1940)
64 Fuji K , and Matsukawa, T *J Pharm Soc Japan* **56**, 158 (1936)
65 Gallagher, T F , and Long W P *J Biol Chem* **162**, 495 (1946), Sorkin, M , and Reichstein, T *Helv Chim Acta* **29**, 1218 (1946)
66 Gallagher, T F , and Xenos J R *J Biol Chem* **165**, 365 (1946)
67 Goldberg M W , and Aeschbacher, R *Helv Chim Acta* **22**, 1185 (1939)
68 Crasshof, H *Z physiol Chem* **223, 249** (1934), **225**, 197 (1934)
69 Hamblen, E C , Cuyler, W K , and Hirst, D V *Endocrinology* **27**, 33 (1940)
70 Hamblen, E C , Cuyler, W K , and Hirst, D V *ibid* **27**, 35 (1940)

71 Hamblen, E C, Cuyler, W K, and Hirst, D V *ibid* **27**, 172 (1940)
72 Hartmann, M, and Locher, F *Helv Chim Acta* **18**, 160 (1935)
73 Hartmann, M, and Wettstein, A *ibid* **17**, 878 (1934)
74 Haskins, A L, Jr *J Clin Endocrinol* **1**, 65 (1941)
75 Haslewood, G A D, Marrian, G F, and Smith, E R *Biochem J* **28**, 1316 (1934)
76 Heard, R D H, Bauld, W S, and Hoffman, M M *J Biol Chem* **141**, 709 (1941)
77 Heard, R D H, Hoffman, M M, and Mack, G E *ibid* **155**, 607 (1944)
78 Heard R D H, and McKay, A F *ibid* **131**, 371 (1939)
79 Hegner, P, and Reichstein, T *Helv Chim Acta* **26**, 715 (1943)
80 Hegner, P and Reichstein, T *ibid* **26**, 721 (1943)
81 Hirschmann, H *J Biol Chem* **136**, 483 (1940)
82 Hirschmann, H *ibid* **140**, 797 (1941)
83 Hirschmann, H *ibid* **150**, 363 (1943)
84 Hirschmann, H, and Hirschmann, F B *ibid* **157**, 601 (1945)
85 Hoehn, W M, and Mason, H L *J Am Chem Soc* **60**, 1493 (1938)
86 Hoehn, W M, and Mason, H L *ibid* **62**, 569 (1940)
87 Hoffman, M M *Can Med Assoc J* **47**, 424 (1942)
88 Hoffman, M M, and Browne, J S L *Federation Proc* **1**, 41 (1942)
89 Hoffman, M M, Kazmin, V E, and Browne, J S L *J Biol Chem* **147**, 259 (1943)
90 Horwitt, B N, Dorfman, R I, Shipley, R A, and Fish, W R *ibid* **155**, 213 (1944)
91 Huebner, C F, Overman, R S, and Link, K P *ibid* **155**, 615 (1944)
92 Inhoffen H H, and Hohlweg, W *Naturwissenschaften* **26**, 96 (1938), Inhoffen, H H, *Angew Chem* **51**, 173 (1938), Inhoffen, H H, Logemann, W, Hohlweg, W and Serini, A, *Ber* **71**, 1024 (1938), Inhoffen, H H, and Kosten, H *ibid* **72**, 595 (1939)
93 Jones, G E S, and Te Linde, R W *Am J Obstet Gynecol* **41**, 682 (1941)
94 Kathol J Logemann, W, and Serini, A *Naturwissenschaften* **25**, 682 (1937)
95 Kimura, T, and Sugiyama, G *J Biochem Japan* **29**, 409 (1939), *Chem Abstracts* 34, 5851[8] (1940)
96 King, L C *J Am Soc* **66**, 1612 (1944)
97 Klyne, W *Biochem J* **40**, Proc lv (1946)
98 Klyne W, and Marrian, G F *ibid* **39**, Proc xlv (1945)
99 Kochakian, C D, Haskins, A L, Jr, and Bruce, R A *Am. J Physiol* **142**, 326 (1944)
100 Koechlin, B, and Reichstein, T *Helv Chim Acta* **27**, 549 (1944)
101 Kuizenga, M H Nelson, J W, Lyster, S C, and Ingle, D J *J Biol Chem* **160**, 15 (1945)
102 Leathem, J H, and Crafts, R C *Endocrinology* **27**, 283 (1940)
103 Loewe, S, and Voss, H E *Arch Gynäkol* **158**, 364 (1934)
104 Longwell, B B, and Gassner, F X *Federation Proc* **6**, 272 (1947)
105 Mamoli, L *Ber* **71**, 2701 (1938)
106 Mamoli, L., Koch, R, and Teschen, H *Z physiol Chem* **261**, 287 (1939)
107 Marker, R E *J Am Chem Soc* **60**, 2442 (1938)
108 Marker, R E *ibid* **61**, 1287 (1939)
109 Marker, R E *ibid* **62**, 2543 (1940)
110 Marker, R E *ibid* **62**, 2621 (1940)
111 Marker, R E *ibid* **62**, 3350 (1940)

112 Marker, R E, and Crooks, H M, Jr *ibid* 64, 1280 (1942)
113 Marker, R. E, Crooks, H. M, Jr, Jones, E M, and Shabica, A C *ibid* 64, 1276 (1942)
114 Marker R. E, Crooks, H M, Jr, and Wagner, R B *ibid* 64, 210 (1942)
115 Marker, R E, Crooks, H. M, Jr, and Wagner, R. B *ibid* 64, 213 (1942)
116 Marker, R E, Crooks, H M, Jr, and Wagner, R. B *ibid* 64, 817 (1942)
117 Marker, R E, Crooks, H M, Jr, Wagner, R. B, Shabica, A C, Jones, E M, and Wittbecker, E L *ibid* 64, 822 (1942)
118 Marker, R. E, Crooks, H M, Jr, and Wittbecker, E L *ibid* 63, 777 (1941)
119 Marker, R. E, Jones, E M, and Krueger, J *ibid* 62, 2532 (1940)
120 Marker, R. E, and Kamm, O *ibid* 59, 1373 (1937)
121 Marker, R E, Kamm, O, Crooks, H M, Jr, Oakwood, T S, Lawson, E J, and Wittle, E. L *ibid* 59, 2297 (1937)
122 Marker, R. E, Kamm, O, Crooks, H M, Jr, Oakwood, T S, Wittle, E L and Lawson, E J *ibid* 60, 210 (1938)
123 Marker, R. E, Kamm, O, Jones, D M, Oakwood, T S *ibid* 59, 614 (1937)
124 Marker, R. E, Kamm, O, and McGrew, R V *ibid* 59, 616 (1937)
125 Marker, R E, Kamm, O, Oakwood, T S, Wittle, E L, and Lawson, E J *ibid* 60, 1061 (1938)
126 Marker, R. E, Kamm, O, and Wittle, E L *ibid* 59, 1841 (1937)
127 Marker, R. E, Kamm, O, Wittle, E L, Oakwood, T S, and Lawson, E J *ibid* 60, 1067 (1938)
128 Marker, R. E., Kamm, O, Wittle, E. L, Oakwood, T S, Lawson, E J, and Laucius, J F *ibid* 59, 2291 (1937)
129 Marker, R E, and Krueger, J *ibid* 62, 79 (1940)
130 Marker, R. E, and Lawson, E J *ibid* 60, 2928 (1938)
131 Marker, R. E, and Lawson, E J *ibid* 61, 588 (1939)
132 Marker, R. E, Lawson, E J, Wittle, E L, and Crooks, H M, Jr *ibid* 60, 1559 (1938)
133 Marker, R. E, and Rohrmann, E *ibid* 60, 1565 (1938)
134 Marker, R E, and Rohrmann, E *ibid* 61, 2537 (1939)
135 Marker, R. E, and Rohrmann, E *ibid* 61, 3476 (1939)
136 Marker, R. E, and Rohrmann, E *ibid* 61, 3592 (1939), 62, 518, 521 (1940)
137 Marker, R. E, and Rohrmann, E *ibid* 62, 898 (1940)
138 Marker, R. E, Rohrmann, E, Crooks, H M, Wittle, E L, Jones, E M, and Turner, D L. *ibid* 62, 525 (1940)
139 Marker, R. E, Rohrmann, E, and Jones, E M *ibid* 62, 648 (1940)
140 Marker, R E, Rohrmann, E, Lawson, E J., and Wittle, E L *ibid* 60, 1901 (1938)
141 Marker, R E, Rohrmann, E, and Wittle, E L *ibid* 60, 1561 (1938)
142 Marker, R E, Rohrmann, E, Wittle, E L, Crooks, H M, Jr, and Jones E J. *ibid* 62, 650 (1940)
143 Marker, R. E, Tsukamoto, T, and Turner, D L *ibid* 62, 2525 (1940)
144 Marker, R E, and Turner, D L *ibid* 62, 2540 (1940)
145 Marker, R. E, and Turner, D L *ibid* 62, 3003 (1940)
146 Marker, R E, and Wagner, R. B *ibid* 64, 216 (1942)
147 Marker, R. E, and Wittle E L *ibid* 61, 855 (1939)
148 Marker, R E, and Wittle, E L *ibid* 61, 1329 (1939)
149 Marker, R. E, Wittle, E L., and Lawson, E J *ibid* 60, 2931 (1938)
150 Marker, R. E, Wittle, E L., and Plambeck, L *ibid* 61, 1333 (1939)
151 Marrian, G F *Biochem J* 23, 1090 (1929)

152 Marrian, G F *ibid* **40**, Proc xix (1946)
153 Marrian, G F, and Gough, N *Nature* **157**, 438 (1946), *Biochem J* **40**, 376 (1946)
154 Mason, H L, Hoehn, W M, McKenzie, B F, and Kendall, E C. *J Biol. Chem* **120**, 719 (1937)
155 Mason, H L, and Kepler, E J *ibid* **161**, 235 (1945)
156 Masson G, and Hoffman, M M *Endocrinology* **37**, 111 (1945)
157 Mazer C, and Goldstein, L Clinical Endocrinology in the Female Saunders, Philadelphia 1932
158 *Meystre, Ch, Ehmann, L, Neher, R, and Miescher, K Helv Chim Acta* **28**, 1252 (1945)
159 Meystre, Ch, Frey, H, Neher, R Wettstein, A, and Miescher, K *ibid* **29**, 627 (1946)
160 Meystre, Ch, Frey, H Wettstein, A, and Miescher, K *ibid* **27**, 1815 (1944),
161 Meystre, Ch and Miescher K *ibid* **28**, 1497 (1945)
162 Meystre, Ch, and Miescher, K *ibid* **29**, 33 (1946)
163 Miescher, K, Hunziker, F, and Wettstein, A *ibid* **23**, 400, 1367 (1940)
164 Miescher, K, and Kägi, H *ibid* **22**, 184 (1939)
165 Moffett, R B, Stafford, J E, Linsk, J, and Hoehn, W M *J Am Chem. Soc* **68**, 1847 (1946)
166 Morsman, H, Steiger, M, and Reichstein, T *Helv Chim Acta* **20**, 3 (1937)
167 Muller, H A *Klin Wochschr* **19**, 318 (1940)
168 Mussio-Fournier, J C, Morato-Manaro, J, and Albrieux, A *Arch clin inst endocrinol Montevideo* **1**, 50 (1937–40)
169 Odell, A, and Marrian, G F *Biochem J* **30**, 1533 (1936)
170 Odell A D, and Marrian, G F *J Biol Chem* **125**, 333 (1938)
171 Oppenauer R V *Rec trav chim* **56**, 137 (1937), Inhoffen, H H, Logemann, W, Hohlweg, W, and Serini, A, *Ber* **71**, 1032 (1938)
172 Pearlman W H *J Biol Chem* **166**, 473 (1946)
173 Pearlman, W H, and Pincus, G *Federation Proc* **1**, 66 (1942)
174 Pearlman, W H, and Pincus, G *ibid* **5**, 79 (1946)
175 Pearlman, W H, Pincus, G, and Werthessen, N T *J Biol Chem.* **142**, 649 (1942)
176 Pfiffner, J J, and North H B *J Biol Chem* **132**, 461 (1940), **139**, 855 (1941)
177 Pincus G and Pearlman, W H *Vitamins and Hormones* **1**, 294 (1943)
178 Plattner Pl A Heusser, H, and Angliker, E *Helv Chim Acta* **29**, 468 (1946)
179 Plattner Pl A, Ruzicka L, Heusser, H, and Angliker, E *ibid* **30**, 385 (1947)
180 Plattner, Pl A and Schreck, W *ibid* **24**, 472 (1941)
181 Reich H, and Reichstein, T *ibid* **26**, 2102 (1943)
182 Reichstein T, and Fuchs, H G *ibid* **23**, 684 (1940)
183 Reichstein T, and Reich, T *Ann Rev Biochem* **15**, 155 (1946)
184 Rosenheim, O, and Starling W W *J Chem Soc* 377, (1937)
185 Ruzicka, L, and Fischer, W H *Helv Chim Acta* **20**, 1291 (1937)
186 Ruzicka L, and Hofmann K *ibid* **20**, 1281 (1937)
187 Ruzicka, L, Hofmann, K, and Meldahl, H F *ibid* **21**, 371 (1938)
188 Ruzicka L, and Meldahl H F *ibid* **22**, 421 (1939)
189 Ruzicka, L, and Meldahl, H F *ibid* **23**, 364 (1940)
190 Ruzicka L, and Prelog V *ibid* **26**, 975 (1943)
191 Ruzicka L, and Rosenberg H R *ibid* **19**, 357 (1936)
192 Salmon U J, Geist, S H, and Salmon, A A *Proc Soc Exptl Biol Med* **47** 279 (1941)

193 Salmon, U J, and Salmon, A. A *ibid* **43**, 709 (1940)
194 Sawlewicz, J, and Reichstein, T *Helv Chim Acta* **20**, 992 (1937)
195 Schiller, S, Miller, A M, Dorfman, R. I, Sevringhaus, E L, and McCullagh, E P *Endocrinology* **37**, 322 (1945)
196 Schoenheimer, R, and Berliner, F *J Biol Chem* **115**, 19 (1936)
197 Schueler, F W *Endocrinology* **38**, 401 (1946)
198 Schwenk, E and Whitman B U S Patent 2 224 968 June 10, 1941
199 Selye H *J Pharmacol* **71**, 236 (1941), Selye, H, and Stone, H J *ibid* **80**, 386 (1944)
200 Selye, H *Endocrinology* **30**, 437 (1942)
201 Serono C, and Marchetti, L *Gazz chim ital* **72**, 151 (1942), *Chem Abstracts* **37**, 2742 (1943)
202 Shoppee, C W and Reichstein, T *Helv Chim Acta* **24**, 351 (1941)
203 Slotta, K H, Ruschig, H, and Fels, E *Ber* **67**, 1270 (1934)
204 Smith, E R., Hughes D, and Haslewood, G A. D *Nature* **132**, 102 (1933)
205 Smith, G V S, and Kennard, J H *Proc Soc Exptl Biol Med* **36**, 508 (1937)
206 Spielman, M A, and Meyer, R. K. *J Am Chem Soc* **61**, 893 (1939)
207 Stavely, H E *ibid* **63**, 3127 (1941)
208 Steiger, M, and Reichstein, T *Helv Chim Acta* **20**, 817 (1937)
209 Steiger, M, and Reichstein, T *ibid* **21**, 161 828 (1938)
210 Strickler, H S, Walton, M E, and Wilson, D A. *Proc Soc Exptl Biol Med* **48**, 37 (1941)
211 Sutherland E S, and Marrian G F *Biochem J* **40**, Proc lxi (1946)
212 Tavastsherna N I *Arch sci biol U S S R* **40**, 141 (1936), *Chem Abstracts* **31**, 6670 (1937)
213 Turfitt G E *Biochem J* **40**, 79 (1946)
214 Turner, R B *J Am Chem Soc* **69**, 726 (1947)
215 von Auwers, K *Ann* **420**, 84 (1920) Skita A *Ber* **53**, 1792 (1920)
216 Van Heuverswyn, J, Collins V J, Williams, W L., and Gardner, W U *Proc. Soc Exptl Biol Med* **41**, 552 (1939)
217 Venning E H *Endocrinology* **39**, 203 (1946)
218. Venning E H, and Browne, J S L *Proc Soc. Exptl Biol Med* **34**, 792 (1936).
219 Venning E H, and Browne, J S L *Endocrinology* **21**, 711 (1937)
220 Venning E H, and Browne, J S L *ibid* **27**, 707 (1940)
221 Wallis, S, and Fernholz E *J Am Chem Soc* **57**, 1504 (1935)
222 Westphal, U *Naturwissenschaften* **24**, 696 (1936)
223 Westphal, U *Z physiol Chem* **273**, 13 (1942)
224 Westphal, U, and Buxton C L *Proc Soc Expt Biol Med* **42**, 749 (1939).
225 Westphal, U, and Schmidt-Thomé *Ber* **69**, 889 (1936)
226 Wettstein, A. *Helv Chim Acta* **23**, 388 (1940)
227 Wettstein, A **23**, 1371 (1940), *ibid* **24**, 311 (1941)
228 Wettstein, A, and Hunziker, F *ibid* **23**, 764 (1940)
229 Willstatter R, and Mayer, E W *Ber* **41**, 2199 (1908)
230 Windaus A *ibid* **49**, 1724 (1916)
231 Wintersteiner, O, and Allen W M *J Biol Chem* **107**, 321 (1934)
232 Yarnall, W A, and Wallis E S *J Am Chem Soc* **59**, 951 (1937), J Org. Chem **4**, 270 (1939)
233 Zondek, B *Nature* **143**, 282 (1939) Investigations on the Genital Functions and their Regulations Williams and Wilkins, Baltimore, 1941

Chapter XII

Biochemistry of Androgens

By RALPH I DORFMAN

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I. Sources of Androgens

As a result of detailed investigations involving sometimes delicate bioassay methods, sometimes careful clinical observations, and sometimes histological and cytological studies, it has now been established that androgens are produced in at least three tissues and probably in a fourth Well defined pure substances possessing androgenic activity have been isolated and identified from testicular and adrenal tissue Although no pure androgen has been isolated from ovarian tissue, the physiological evidence of its production there seems convincing The placenta may elaborate androgenic material, but the evidence at the present moment seems indecisive

A Testis

The earliest investigators were able to relate the physiological influences of castration and the loss of a biologically active substance, the androgen The first convincing demonstration of androgens in testis came from the laboratory of Professor Koch, where McGee in 1927 (148) *demonstrated androgenic material in lipid extracts of bull testis* Subsequent studies, including isolations of pure androgens from testis tissue, are discussed elsewhere in this chapter (see page 464)

Sufficient evidence is at hand to prove that the androgenic hormone of the testis is produced by the interstitial tissue Thus it is well known that, if the seminal epithelium is caused to atrophy without damage to the interstitial tissue, androgenic material continues to be secreted by the modified testis If an animal is made cryptorchid (158) or the testis treated with adequate dosage of X-rays (207), such a condition is realized Experiments of this sort have been carried out on dogs, horses, pigs, guinea pigs, rabbits, and rats with similar results In spite of the destruction of the seminal epithelium, the accessory genital organs are preserved in a normal functioning state In one experiment extracts of the cryptorchid testis of swine were demonstrated to contain androgenic material when tested on the capon's comb

A second line of proof that interstitial tissue rather than seminal *epithelium is responsible for the elaboration of androgenic material is* found in those experiments in which the interstitial tissue is caused to atrophy while the seminal epithelium remains intact When pitch was *administered to rodents, it was found that the interstitial tissue of the* testis was damaged with a parallel atrophy of the secondary sex glands, in spite of the fact that the seminal epithelium remained normal (9) In another report (159), interstitial damage was produced in rats by feeding the animals a vitamin-B-complex-deficient diet Again the

seminal epithelium remained normal, but the prostate and seminal vesicles atrophied

Finally, it has been shown that certain interstitial cell tumors of the testis in humans produce enormous quantities of androgenic material In one such case Venning *et al* (212) have reported a titer of 1015 mg /day of 17-ketosteroids, which represents an increase of about 10,000%

Evidence, therefore, indicates that the source of the androgens in the testis is the interstitial tissue

B Ovary

In experiments on fat soluble extracts of sow ovarian tissue, it has been shown that such extracts do contain androgenically active material (172) This work is reinforced by the striking experiments of Hill (106–108), who showed that, if ovaries are transplanted to the ears of castrated male mice, androgenic stimulation of the otherwise involuting accessories is observed Here it is of interest to note that, although the ovary normally secretes androgenic material, exteriorizing the organ, as in transplantation to the ear, increases the rate of synthesis of the androgens From the work of Hill it appears that the important factor is that of temperature, since ovaries grafted into the abdomen of castrated males were unable to maintain the accessories In a series of experiments with rats Deanesley (48) confirmed the findings of Hill

C Adrenal Cortex

Androgens in the adrenal cortex have been demonstrated by the direct isolation of androgenic substances from adrenal cortical extracts and by the presence of high concentrations of androgenic material in body fluids in conditions of increased activity of the adrenal cortex, and indirectly by the presence of androgenic material in the complete absence of the gonads

Adrenosterone (XXI) (188,189), androstanediol-3(β),11(β) one-17 (XXIII) (166), Δ^4-androstenedione-3,17 (XXII) (215), and 17-"β"-hydroxyprogesterone (XXIV) (177 178) have been isolated from adrenal cortical extracts (for formulas, see page 532) All four have been shown to possess androgenic activity The possibility still exists that the C_{19} steroids are not present in the gland as such, but rather that they are formed during the process of working up the extracts On the other hand other androgens may be present in the gland which as yet have escaped isolation This may be said since no isolations of adrenal cortical extracts have been carried out with the object of isolating androgens, and therefore important glandular androgens may not yet have been isolated

The administration of adrenotrophic extracts to castrated rats caused the development of the prostate and seminal vesicles (47) The adrenal cortices were greatly enlarged in these animals The effect was absent in adrenalectomized rats but demonstrable in hypophysectomized animals These experiments have been confirmed (165)

Female mice of the strain CE, when spayed at one to three days of age, develop tumors which appear to secrete androgenic material as evidenced by the growth and development of the accessory sex organs The same was true for castrated males of the same strain (221,222)

Masculinization as a result of hyperactivity of the adrenal cortex is well known in women Increases in androgenic material in the urine of such patients are found In 1936 Callow (30) reported the isolation of massive amounts of the androgen, dehydroisoandrosterone, from the urine of a six year-old girl suffering from an adrenal cortical cancer Numerous other similar cases in females have been reported and are discussed elsewhere (page 513)

Ovariectomized women excrete considerable quantities of androgenic material (31), and it has been possible to isolate two androgens, androsterone (I) and dehydroisoandrosterone (III), from the urine of such subjects in amounts only slightly less than that found in the urine of normal women (110)

The castration of week-old rats does not arrest the development of the seminal vesicles and prostate, but instead these organs continue to develop until the fifth week of life (185) If, however, young rats are adrenalectomized and castrated, complete atrophy of the prostate results (11,12)

D Placenta

It has been reported that extracts of human placental tissue contain androgenic material (42) Experiments in the author s laboratory have not confirmed these findings

II Isolation of Androgens and Related Compounds

A Urinary Androgens

Androgens have been studied in the urines of normal individuals as well as of those with various pathological conditions Human urines contain relatively high concentrations of androgens as compared to other species These biologically active substances have been demonstrated in the urine of men and women as well as children of both sexes In addition to these substances, which possess androgenic activity, a number of steroids have been isolated which possess no biological activity, yet are chemically closely related to the androgens In some cases these

biologically inert substances have been shown to be derived from androgenically active material The metabolic considerations are discussed elsewhere (page 517)

Within a period of two years three laboratories were able to show the presence of androgenic material in both normal men's and women's urine Loewe *et al* (128) were able to show that men's urine contained material capable of stimulating the seminal vesicles of castrated mice

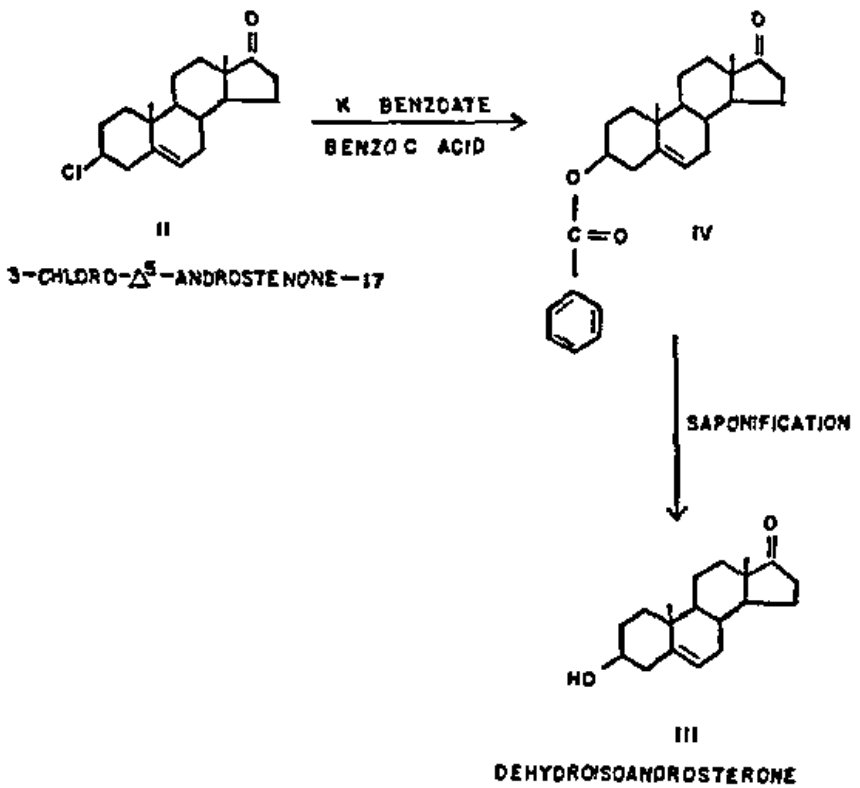


FIG 1 —3-Chloro-Δ^5 androstenone-17 converted to dehydroisoandrosterone

Funk and Harrow (86) and Funk, Harrow, and Lejwa (87) also reported urinary androgens which could be extracted with fat solvents From the Chicago laboratories papers by Womach and Koch (220) and Gallagher and Koch (89) demonstrated that androgens were present in female as well as male urine

B Isolation of Androsterone and Dehydroisoandrosterone

Butenandt (17,18) announced the first isolations of crystalline material from concentrates of men's urine which possessed androgenic activity In a later group of papers (19,20,25), the presence of an androgen with the empirical formula $C_{19}H_{30}O_2$ (I) was demonstrated, as well as two other substances, $C_{19}H_{27}OCl$ (II) and $C_{19}H_{28}O_2$ (III)

The compound $C_{19}H_{30}O_2$ (I) was named androsterone It proved to be saturated since it did not decolorize bromine One oxygen atom

was present as a ketone group, since an oxime, a semicarbazone, and a phenylhydrazone could be prepared The second oxygen atom was present as a secondary alcohol group, since the compound formed a monoacetate, and oxidation with chromic acid yielded a diketone A structure was assigned to the compound which was later shown to be correct by partial synthesis Butenandt and co-workers were able to show the relationship among the three substances isolated

The chloro compound $C_{19}H_{27}OCl$ (II) was found to be physiologically inactive That the compound contained an unsaturated grouping was

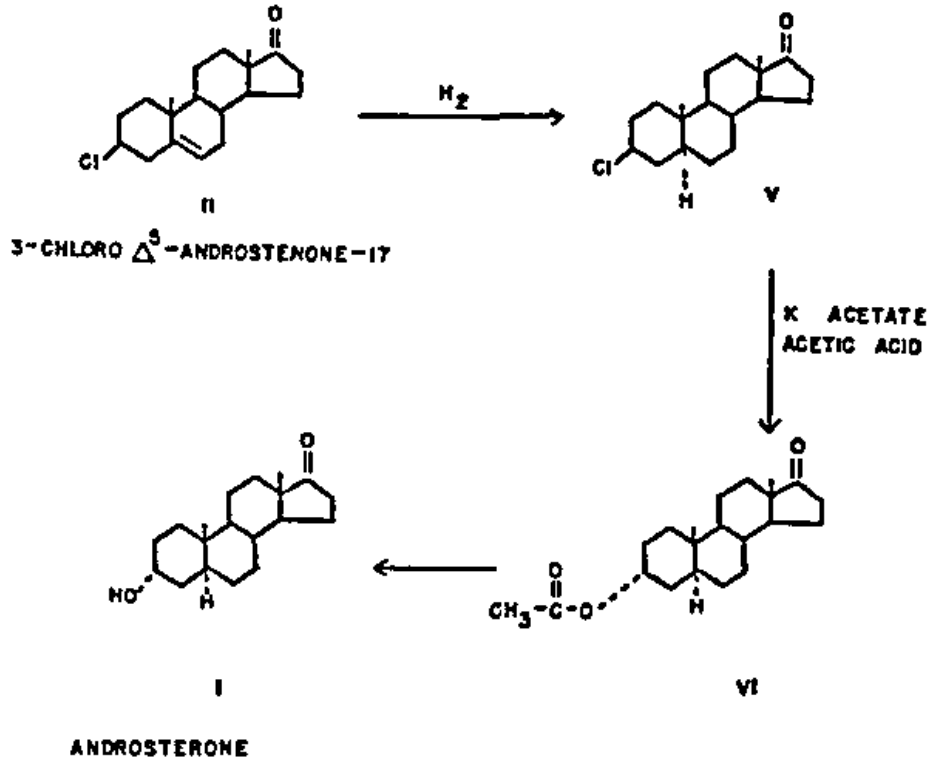


FIG 2 —3-Chloro-Δ^5-androstenone-17 converted to androsterone

shown by the facts that it decolorized bromine, gave a yellow color with tetranitromethane, and took up one mole of hydrogen when subjected to catalytic hydrogenation to form a saturated ketone On treatment of the chloro compound with potassium benzoate and benzoic acid the compound $C_{19}H_{28}O(OOCC_6H_5)$ (IV) was obtained, which on hydrolysis yielded an unsaturated hydroxy ketone $C_{19}H_{28}O_2$ (III) identical with the second androgen isolated (Fig 1) Treatment of the unsaturated hydroxy-ketone $C_{19}H_{28}O_2$ (III), which was biologically active, with hydrochloric acid and heat produced the androgenically inactive chloro compound $C_{19}H_{27}OCl$ (II) Thus it was concluded that the chloro compound was probably an artifact and had been formed during the course of extraction and isolation

C Conversion of Dehydroisoandrosterone to Androsterone

It still remained to show the structural relationship between androsterone and dehydroisoandrosterone (III) The chloro derivative was subjected to catalytic hydrogenation and the saturated chloro derivative was obtained (V) This was treated with potassium acetate and acetic acid to form androsterone acetate (VI), which yielded androsterone (I) on saponification (Fig 2)

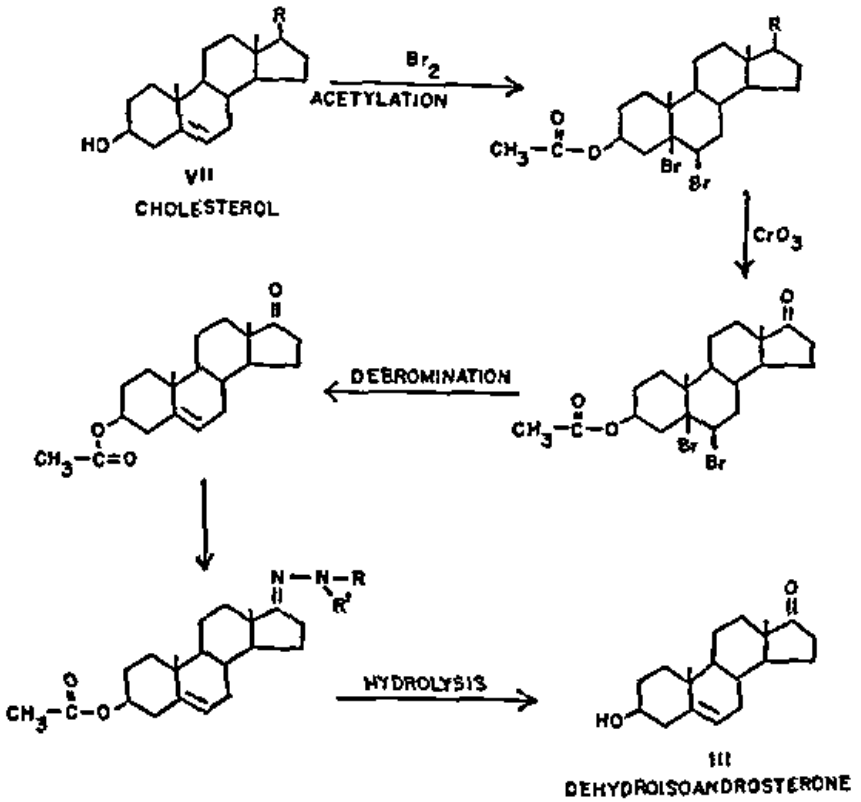


Fig 3 —Partial synthesis of dehydroisoandrosterone from cholesterol

Dehydroisoandrosterone (III) was shown later to have the same steric configuration at C_3 as cholesterol (VII) It was possible to prepare this androgen from cholesterol (VII) The cholesterol was acetylated and brominated The side chain was oxidized with chromic acid to yield the C_{17} ketone which, on removal of the bromine, and hydrolyzing, yielded dehydroisoandrosterone identical with that prepared from the urinary extracts (Fig 3) (19–21,23,25)

The partial synthesis of dehydroisoandrosterone was carried out almost simultaneously by three other groups of workers Thus Ruzicka and Wettstein (196) and Wallis and Fernholz (216a) accomplished the partial synthesis from cholesterol, while Oppenauer (170) was able to convert α sitosterol to dehydroisandrosterone

D Partial Synthesis of Androsterone and Isomers

Clarification of the structure of androsterone was accomplished by Ruzicka's group in 1934 (193), when androsterone and the other three stereoisomers, involving carbon atoms 3 and 5, were prepared by partial synthesis For the synthesis of androsterone, cholesterol was reduced

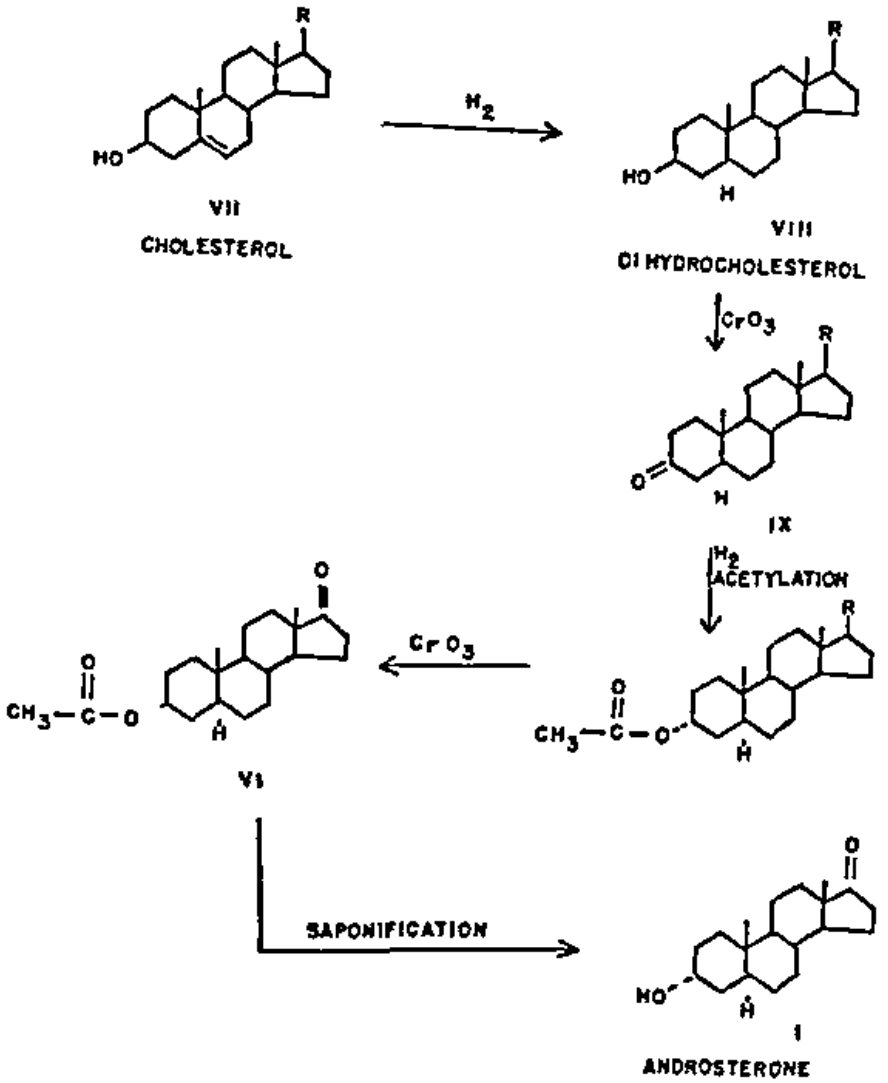


Fig 4 —Partial synthesis of androsterone from cholesterol

to dihydrocholesterol (VIII) which was oxidized in turn to cholestanone (IX) with chromic acid On hydrogenation in acid solution and subsequent acetylation, the cholestanone (IX) was converted into epidihydrocholesterol acetate (X) Oxidation of the side chain with chromic acid yielded androsterone acetate (VI), and on hydrolysis androsterone which was identical with that isolated by Butenandt from the urine was realized (Fig 4)

Ruzicka *et al* (193) also prepared, by partial synthesis from cholesterol, the three additional isomers of androsterone at C-3 and C-5 as illustrated in Fig 5

The partial synthesis of androsterone (I) was also accomplished by Butenandt *et al* from cholesterol (21), by Dirscherl (54) from cinchol, by

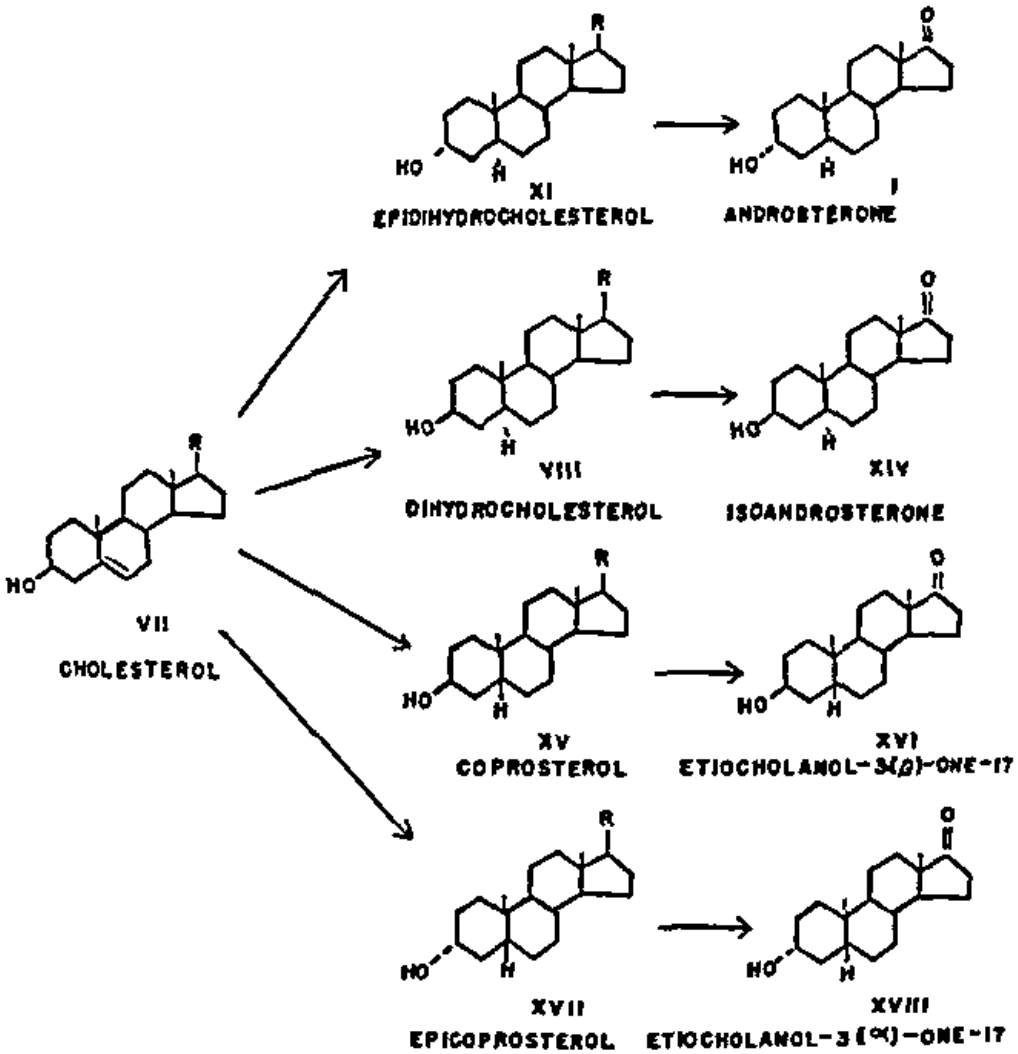


FIG 5.—Schematic representation of partial synthesis of androsterone and three isomers from cholesterol

Dalmer *et al* (43) from sitosterol and stigmasterol, and by Marker (135) and Marker *et al* (137) from cholesterol

E ISOLATION OF TESTOSTERONE FROM TESTIS TISSUE

After the isolation of the two androgens, androsterone and dehydroisoandrosterone, from men's urine it became apparent that the androgenic material in bull testis must be due to still another substance The work of Gallagher and Koch (90) had indicated that the androgenic material

in testis tissue was more labile to alkali than the androgens in urine Secondly, the highly purified fractions from bull testis were more active on a weight basis than the pure compounds isolated from urine Finally, when testis extracts and urinary extracts were administered to castrated rats or mice at equivalent levels in terms of capon units, the testis material proved to be far more active than the urinary extract David *et al* (46) finally were able to isolate an androgen, testosterone, from bull testis which was shown to be approximately six times more active than androsterone (I) on the basis of the capon's comb, and which differed chemically from androsterone (I) Recently David's isolation was confirmed in the laboratory of Ruzicka, where testosterone (XXV) was isolated from the testis of stallions (205)

F Partial Synthesis of Testosterone

The synthesis of testosterone and proof of structure was quickly accomplished by Ruzicka and Wettstein (196) and by Butenandt and Hanisch (21) The partial synthesis as accomplished by Ruzicka and Wettstein consisted of converting cholesterol (VII) by oxidizing its dibromo acetate derivative to dehydroisoandrosterone acetate (IV) The ketone group of dehydroisoandrosterone acetate was reduced to the Δ^5-androstenediol-3(β),17(α)-3-monoacetate (XIX), which was benzoylated to form the 3-acetoxy, 17-benzoxy derivative On partial saponification the acetate group was removed with the formation of Δ^5-androstenediol-3(β),17(α)-17-benzoate (XX) This compound on bromination followed by oxidation, debromination, and saponification yielded testosterone (XXV), which was identical with the compound isolated from testis tissue (Fig 6)

The synthesis of Butenandt and Hanisch is similar to that described by Ruzicka and Wettstein

G Androgens from Adrenal Tissue

Studies on adrenal cortical extracts have yielded four crystalline androgens adrenosterone (XXI), Δ^4-androstenedione-3,17 (XXII), androstanediol-3(β),11-one-17 (XXIII), and 17 "β'-hydroxyprogesterone (XXIV) Although the extracts were subjected to relatively mild chemical treatment, it has been pointed out by Reichstein that these compounds may represent artificial degradation products of C_{21} compounds This does not apply to 17-"β"-hydroxyprogesterone (XXIV)

Adrenosterone (XXI) was isolated from an adrenal cortical extract by Reichstein (188,189) From similar extracts androstanediol-3(β),11-one 17 (XXIII) (215), and Δ^4-androstenedione-3,17 (XXII) (215) were realized The structural considerations of the first two androgens are discussed elsewhere (Chapter XIII) in this volume, and Reichstein and

von Euw (215) as well as Pfiffner and North (178) have reported the isolation of 17"β"-hydroxyprogesterone (XXIV) The constitution of 17"β"-hydroxyprogesterone (XXIV) has been proved by degradation studies (177,178), and partial synthesis has been accomplished

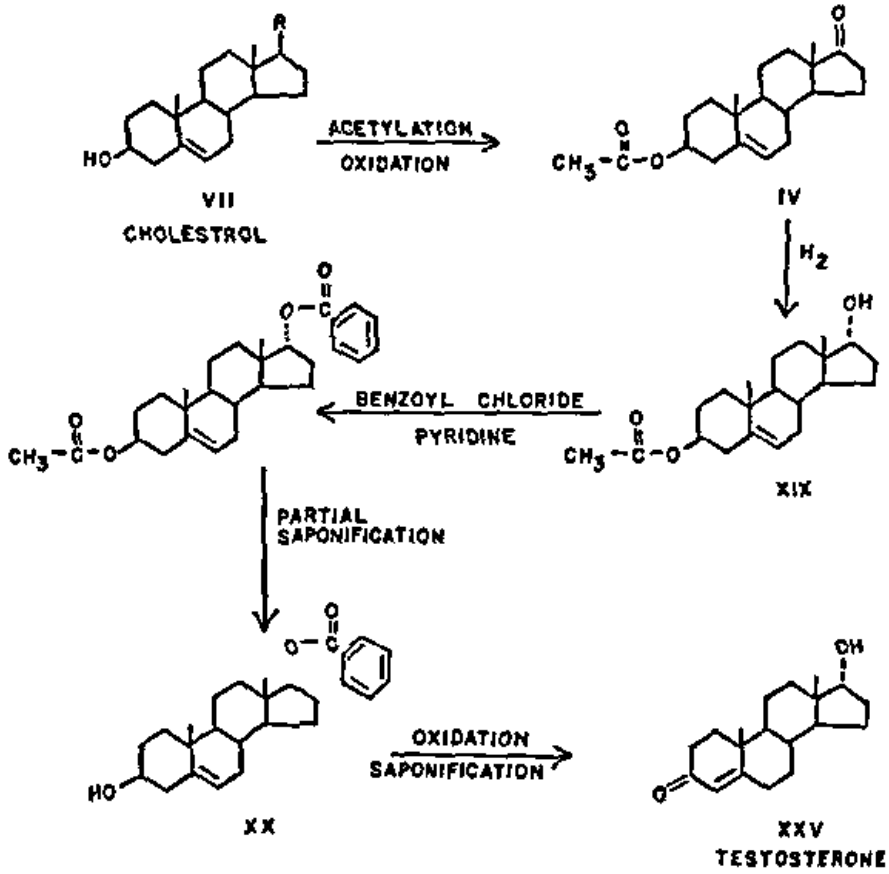


FIG 6 —Partial synthesis of testosterone from cholesterol
Note For cholestrol read cholesterol in VII.

H ISOLATION OF 11 HYDROXYANDROSTERONE

11 Hydroxyandrosterone (XL) was isolated first by Mason (141) and Mason and Kepler (144) from the urines of various patients showing adrenal cortical involvements, such as tumors and hyperplasia The substance has also been isolated from the urine of a female pseudohermaphrodite showing no apparent adrenal involvement by Miller, Dorfman, and Sevringhaus (154) and from normal male urine (142) The former workers found the compound to be androgenic by the chick comb test

The formula $C_{19}H_{30}O_3$ was found by analysis, and on acetylation at 90°C with acetic anhydride and pyridine, the compound yielded a monoacetate The material was not precipitated with digitonin in 90% methanol It formed a yellow dinitrophenylhydrazone, and on oxidation with chromic acid gave a product identical with androstanetrione-3,11,17 (XLI) (Fig 7)

On treatment of the monoacetate with hydrochloric acid and acetic acid an androstenolone (XLII) was obtained, which appeared to be identical with one previously obtained from urine (153)

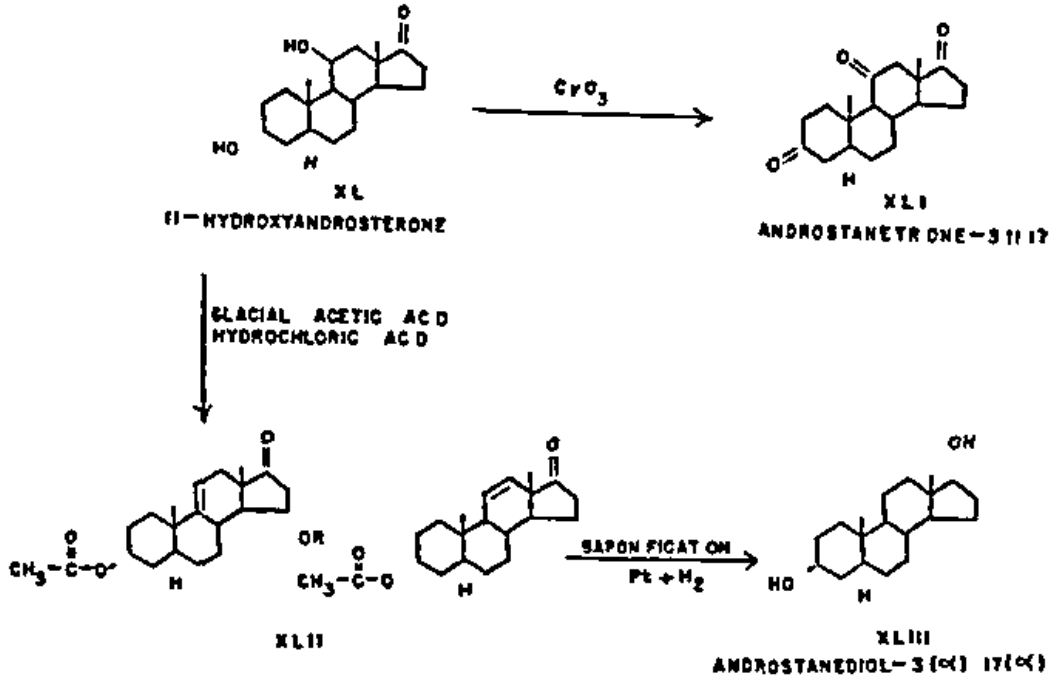


Fig 7 —Proof of structure of 11 hydroxyandrosterone

I Isolation of Δ^5 Androstenetriol-3(β),16,17

Δ^5 Androstenetriol-3(β) 16,17 (XLIV) was first isolated by Hirschmann (111) from the urine of a seven year-old boy with an adenocarcinoma of the left adrenal cortex This steroid was also isolated from normal urine (139) The compound was inactive as an androgen at *doses up to seventy times the amount necessary to produce a significant* response in the chick's comb with androsterone (112) This steroid is of interest, however, particularly because of its relationship to dehydroisoandrosterone (III) This relationship is similar to that found between estrone and estriol Hirschmann was able to prove the structure of this steroid by degrading the compound to a known ketodicarboxylic acid (XLVI), and by the conversion of the monoacetate of the *isolated compound into the known β-3 hydroxy Δ⁵-etiobilienic acid* (XLVIII) Thus the isolated compound, which contained one nuclear double bond, was reduced with hydrogen and palladium to the saturated triol (XLV) The triol was oxidized with chromium trioxide at room temperature to form the ketodicarboxylic acid (XLVI) (Fig 8)

In a second series of reactions, isoandrosterone (XIV) was converted to 16-benzylidinandrostanol-3(β) one-17 (XLVII), with sodium methylate and benzaldehyde This product was acetylated with pyridine and acetic anhydride and oxidized with chromium trioxide, and after saponi-

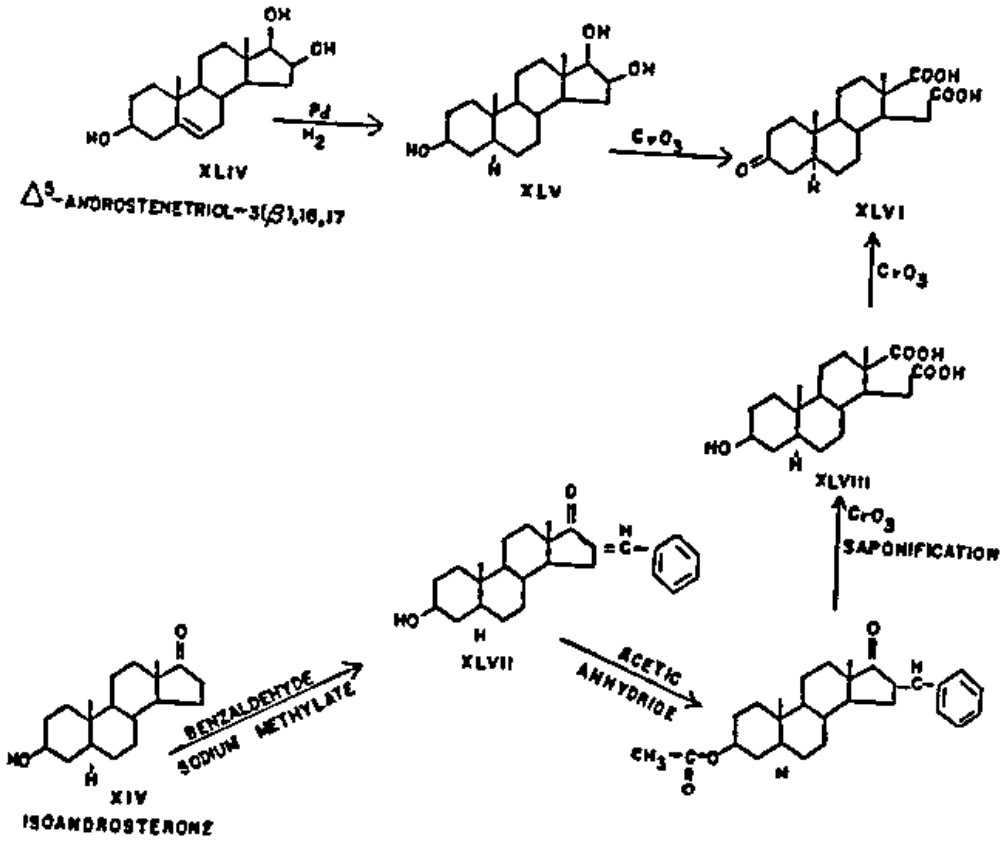


FIG 8—Proof of structure of Δ5 androstenetriol-3(β),16,17

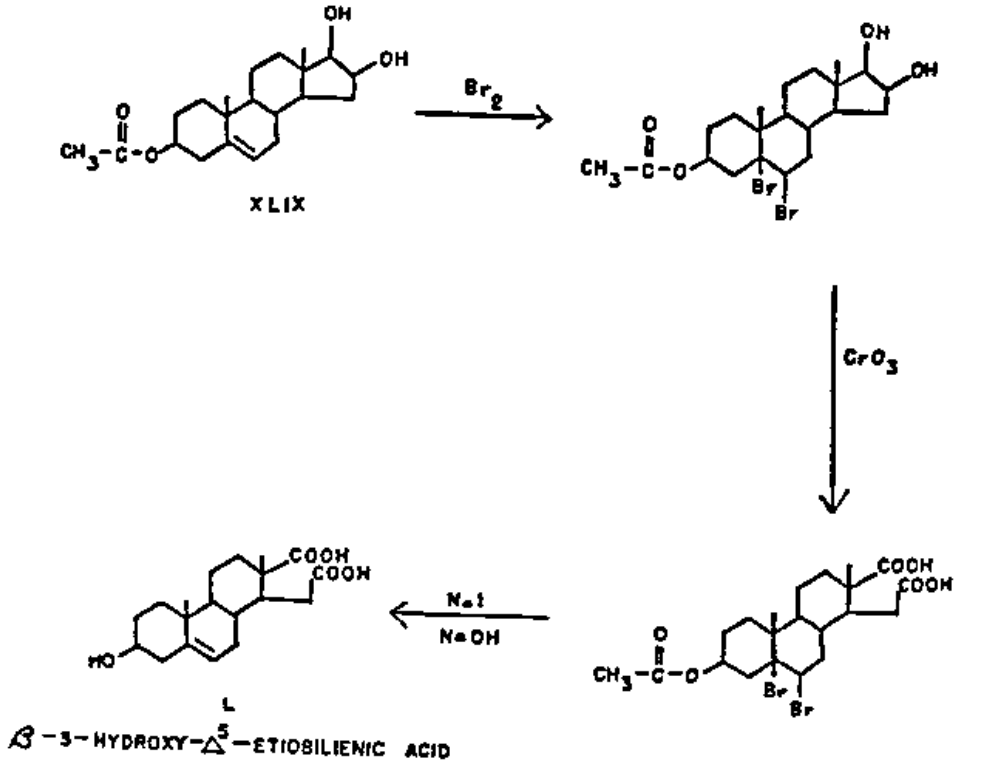


FIG 9—Conversion of Δ5 androstenetriol-3(β),16,17 to β-3-hydroxy-Δ5-etiobilienic acid

fication yielded the β-3-hydroxyetioallobilianic acid (XLVIII) The free β-3 hydroxyetioallobilianic acid (XLVIII) was oxidized with chromic acid to 3-ketoetioallobilianic acid (XLVI), this proved to be identical with that prepared from the isolated compound (Fig 7)

From the above evidence it was apparent that the compound must be androstenetriol-3,16,17 The fact that the compound was precipitated with digitonin was indicative of a 3(β) hydroxy configuration Comparison of the optical rotations of androstenetriol triacetate and androstanetriol triacetate indicated that the doub'e bond was between C-5 and C-6 Further evidence for the 3(β)-hydroxy and Δ^5 was found by a third set of reactions The 3-monoacetate of the isolated triol was brominated and oxidized with chromium trioxide The reaction product was debrominated with sodium iodide, and β-3 hydroxy-Δ^5-etiobilienic acid (L) was isolated, which was identical with a known sample (Fig 9)

J C_{21} Compounds from Testis Tissue

Marker *et al* (136,138) have demonstrated that, although the bull excretes sizable amounts of such C_{21} steroids as pregnanediol-3(α),20(α) (LXXVII, page 432), allopregnanediol-3(α),20(α) (CII) and allopregnanediol 3(β),20(α) (LXXXIX, page 435), the steer does not This would indicate that the testis is capable of producing some C_{21} steroids The work of Ruzicka and Prelog (194) has demonstrated the presence of two C_{21} compounds in swine testis, namely Δ^5-pregnenol-3(β)-one-20 (LIII, page 422) and allopregnanol-3(β)-one-20 (LIV, page 422)

K Isolation of Δ^{16}-Androstenol-3(α) and Δ^{16}-Androstenol-3(β)

Prelog *et al* (182) isolated from the lipid extracts of swine testis two isomeric androstenols, Δ^{16} androstenol-3(α) (LV) and Δ^{16}-androstenol-3(β) (LVI) Both compounds had a musk-like odor Their structures were established by partial synthesis (184) Androstanol 17(β)-one-3 hexahydrobenzoate (LVII) was heated to 300°C in an atmosphere of nitrogen to form Δ^{16}-androstenone 3 (LVIII) The latter compound was reduced with aluminum isopropylate (Meerwein-Pondorf) to form the two C-3 epimers, which in turn were separated with digitonin (Fig 10)

L An Androstanol-3(β)-one from Pregnant Mare Urine

Heard and McKay (102) isolated a digitonin-precipitable neutral steroid from the urine of pregnant mares which had the formula $C_{19}H_{30}O_2$ The compound yielded androstane when reduced by the Clemmensen method, and a diketone on oxidation with chromic oxide which was not

identical with androstanedione-3,17 (LXX, page 522) The authors feel that the most likely position for the carbonyl group is at C-6 or C-15 (103)

M Miscellaneous Substances Isolated from Swine Testis

Hirano (109) isolated a substance from swine testis which was physiologically inactive, and had the formula $C_{21}H_{32}O_3$ and which he called testalolone He suggested that the structure of this C_{21} compound was either allopregnanol-3(β) one-20-al 21 (LI) or pregnanol 3(β)-one-20-al-21 (LII) However, synthesis of both these compounds by Ruzicka

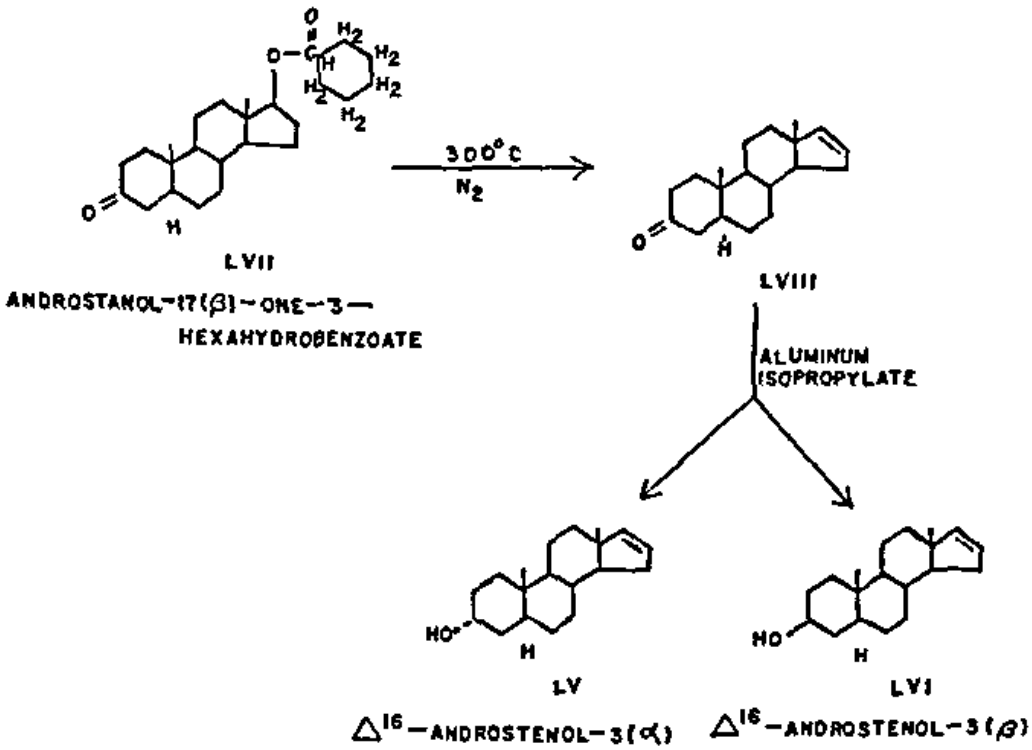


Fig 10.—Partial synthesis of Δ^{16} androstenol-3(α) and Δ^{16} androstenol-3(β) from androstanol 17(β)-one-3

et al (195) has shown this not to be the case Ruzicka and Prelog (194) were able to isolate testalolone with melting point at 268°C, which analyzed for $C_{21}H_{32}O_3$, as compared to Hirano's melting point of 258°–264°C The compound gave a negative test with tetranitromethane and reduced ammoniacal silver nitrate slowly The compound also showed mutarotation

A second compound was isolated from swine testis by Hirano (109) named testriol, which had the formula $C_{19}H_{40}O_3$ and a melting point of 65–66°C This compound has also been isolated from swine testis by

Prelog, Ruzicka, and Steinmann (183) and has been shown to be chimyl alcohol

N Summary of Androgens and Related Substances

The various androgens and related compounds isolated from natural sources are represented in Fig 11 and Table I The androgenic activi-

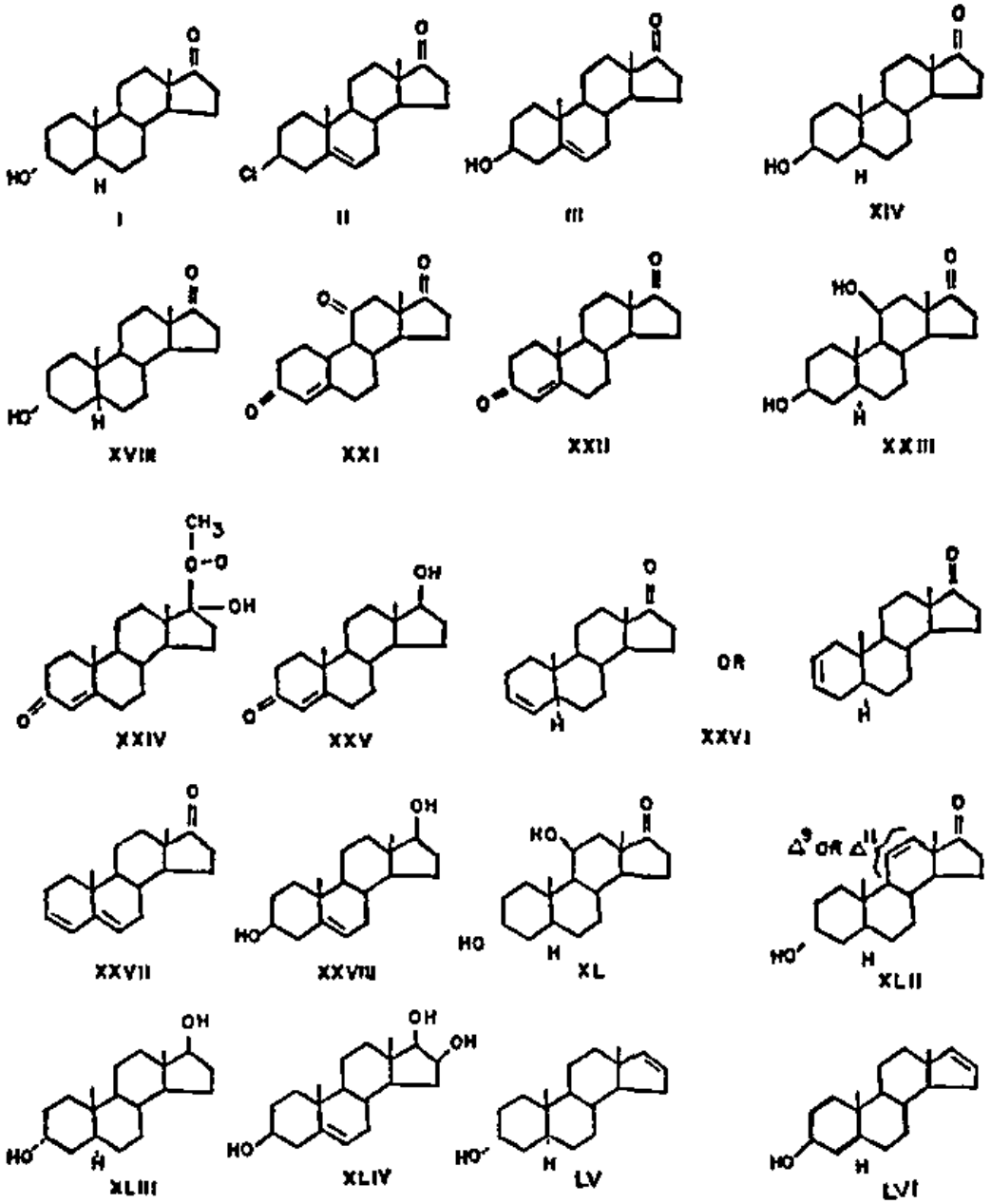


Fig 11 —Androgens and related compounds isolated from natural sources

ties represented are merely approximations on the basis of the capon's comb test These would not necessarily be similar to relative activities derived from mammalian bioassays

TABLE I

ANDROGENS AND RELATED SUBSTANCES ISOLATED FROM NATURAL SOURCES

Compound number	Systematic name	Common name	Source	Approx am t equal to 1 I U
I	Androstanol-3(α) one-17	Androsterone	Human preg cow and bull urine	100
II	3 Chloro-Δ^5 andro sterone 17		Human urine (prob ably artifact)	Inactive
III	Δ^5 Androstenol 3(β) one 17	Dehydroisoan drosterone	Human preg cow, and bull urine	300
XIV	Androstanol-3(β) one-17	Isoandrosterone	Human and preg mare urine	700
XVIII	Etiocholanol-3(α) one-17		Human urine	Inactive at 1200
XXI	Δ^4-Androstenetrione 3 11 17	Adrenosterone	Adrenal cortex	500
XXII	Δ^4-Androstenedione 3 17		Adrenal cortex	100
XXIV	Δ^4-Pregnenol 17(β) dione-3 20	17 β hydroxy progesterone	Adrenal cortex	500
XXV	Δ^4-Androstenol 17(α) one-3	Testosterone	Bull and stallion testis	15
XXVI	$\Delta^{2 \text{ or } 3}$ Androstenone-17		Human urine (prob ably artifact)	1000
XXVII	$\Delta^{3\,5}$ Androstadienone 17		Human urine (prob ably artifact)	400
XXVIII	Δ^5 Androstenediol 3(β) 17(α)		Path human urine	
XL	Androstanediol-3(α) 11-one 17	11 Hydroxy androsterone	Human urine	300
XLII	$\Delta^{9 \text{ or } 11}$ Androstenol 3(α)-one-17		Path human urine (probably artifact)	300
XLIII	Androstanediol-3(α) 17(α)		Human urine?	20
XLIV	Δ^5 Androstenetriol 3(β) 16 17		Human urine	Negative
LV	Δ^{16} Androstanol-3(α)		Boar testis	
LVI	Δ^5-Androstanol-3(β)		Boar testis	

III Form in Which Androgens Occur in Urine

Androgens in urine occur in a water soluble, biologically inactive form On treatment with acid and heat the water soluble complex is split, yielding the fat soluble water insoluble androgen which now is biologically active However the process of hydrolyzing the water soluble complex in addition to liberating the androgens, also causes structural

changes, to some extent, in the biologically active steroids, which tend to decrease the total androgenic activity

As early as 1929, Funk *et al* (87) were able to show that urine extracted after acidification gave larger amounts of active material than untreated urine Adler (1) was able to show that butanol extracts of male urine which were inactive by the capon's comb test could be converted to biologically active material by heating with trichloroacetic acid Other workers confirmed and extended these findings (90 175)

A Isolation of Sulfate Esters

The work of early investigators has been confirmed by the isolation of dehydroisoandrosterone (III) in the form of its sulfate ester from men's urine (162) and the isolation of androsterone sulfate (CIII page 487) from the urine of a patient with an interstitial cell tumor of the testis (212)

In the procedure for the isolation of dehydroisoandrosterone sulfate, the urine was first extracted with *n* butanol (162) The combined butanol extracts were treated with cold sodium bicarbonate and with sodium hydroxide The butanol solution was subjected to repeated extraction with water The aqueous extracts were treated with semicarbazide and a semicarbazone was isolated which analyzed well for the semicarbazone of sodium dehydroisoandrosterone sulfate Hydrolysis of the derivative with hydrochloric acid yielded a substance which, after sublimation and benzoylation, was found to be identical with dehydroisoandrosterone benzoate (CIV)

The isolation of androsterone sulfate by Venning *et al* (212) consisted in a preliminary extraction of the urine with benzene to remove free steroids This was followed by exhaustive extraction with *n* butanol at pH 1, to remove the conjugates The combined butanol extracts were neutralized and extracted with an aqueous solution of sodium hydroxide The butanol extract was neutralized and evaporated to dryness The residue was dissolved in ethanol and after removal of the ethanol insoluble material, the solution was evaporated to dryness The residue was finally dissolved in water and precipitated with acetone After repeated precipitation followed by chromatographic separation, a crystalline conjugated 17 ketosteroid was obtained which analyzed for the sodium salt of androsterone sulfate Proof for the structure of the conjugate was obtained by hydrolysis to the free steroid The conjugate was refluxed for six hours in the presence of hydrochloric acid After hydrolysis, both Δ^2 and Δ^3 androstenone-17 (XXVI) and androsterone (I) were obtained

B Artifacts in Urinary Extracts

A certain number of isolated androgens and related steroids may be considered more as artifacts than as normal urinary constituents The production of artifacts may distort the true picture of metabolites in the urine in three ways First, when a true metabolite is modified, the concentration of this metabolite is decreased, second, the decrease in the concentration of the metabolite may be reflected in the formation of a substance or substances not originally present, and third, the change in the metabolite may result in the production of a second metabolite, thus causing the concentration of a metabolite to increase only as a result of the methods employed These artifacts may be classified as artifacts of degradation, artifacts of substitution, and artifacts of dehydration

1 *Artifacts of Degradation*

No clear cut evidence has been presented to show that androgens or 17-ketosteroids may arise as a result of degradation From the works

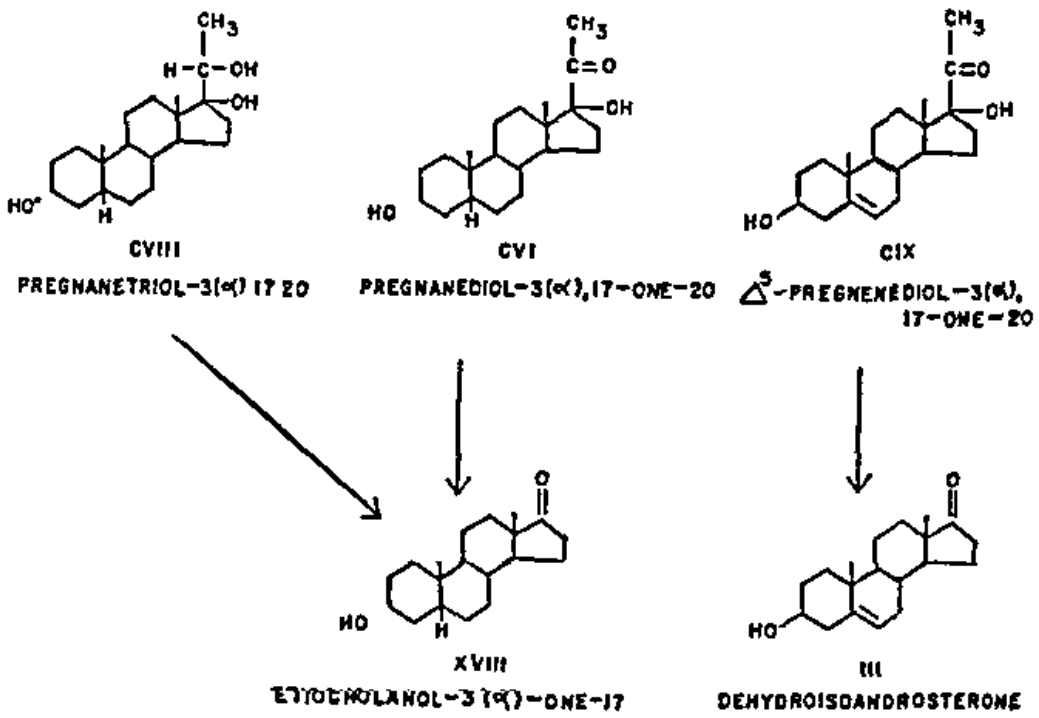
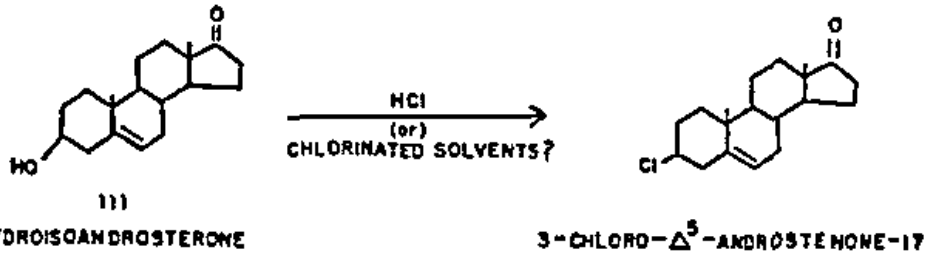


FIG 12 —Possible artifacts of degradation

of Talbot and Eitingon (208) as well as from the isolation studies of Butler and Marrian (26), Hirschmann and Hirschmann (113), and Lieberman and Dobriner (126), it is clear that human urines contain C_{21} compounds with 17-hydroxyl groups The specific compounds isolated are pregnanetriol 3(α) 17,20 (CVIII), pregnanediol-3(α),17-one-20 (CVI),

and Δ^5-pregnenediol-3(β),17-one-20 (CIX) If these compounds are degraded to 17-ketosteroids during the usual acid and heat treatment, we can expect the former two compounds to increase the titer of etiocholanol-3(α)-one-17 (XVIII), while the latter compound would be converted to dehydroisoandrosterone (II) (Fig 12)



III
DEHYDROISOANDROSTERONE

3-CHLORO-Δ^5-ANDROSTENONE-17

FIG 13 —Artifacts of substitution

2 *Artifacts of Substitution*

Artifacts of substitution are those in which hydroxy groups are replaced with chlorine to form the chloro derivative Thus Butenandt and Dannenbaum (18) isolated 3 chloro-Δ^5 androstenone-17 (II) from urinary extracts The fact that this substance is an artifact was suggested by Butenandt. Venning *et al* (212) were able to convert dehydroisoandrosterone sulfate (CV, page 485) to 3-chloro-Δ^5-androstenone-17 (II) by hydrolysis with hydrochloric acid (Fig 13)

3 *Artifacts of Dehydration*

In the category of dehydrations, we have $\Delta^{2 \text{ or } 3}$-androstenone-17 (XXVI), $\Delta^{2,5}$ androstadienone-17 (XXVII), and $\Delta^{9 \text{ or } 11}$-androstenol-3($\alpha$)-one-17 (XLII) (Fig 14) Proof that $\Delta^{2 \text{ or } 3}$-androstenone-17 (XXVI) is a hydrochloric acid artifact of androsterone has been produced by the conversion of a portion of androsterone sulfate (CIII, page 485) to this unsaturated steroid (212) Whether a portion of this sterone is present in urine as such is not known

$\Delta^{2,5}$ Androstadienone-17 (XXVII) appears to arise at least in part by the dehydration of dehydroisoandrosterone (III) (55,181) The latter workers used the characteristic spectrum as a means of identifying the dehydration product They also claim from similar evidence that the compound is present in extracts of unhydrolyzed urines

A third androgen of urine, isolated thus far only from the urine of a girl with an adrenal cancer (218) and from that of a female pseudohermaphrodite (70), is $\Delta^{9 \text{ or } 11}$-androstenol-3($\alpha$)-one-17 (XLII), which appears to be an artifact It has been shown that dehydration of 11 hydroxyandrosterone (XL) with a mixture of hydrochloric and acetic acids

yields an androstenolone which appears to be identical with that isolated from the urine (140)

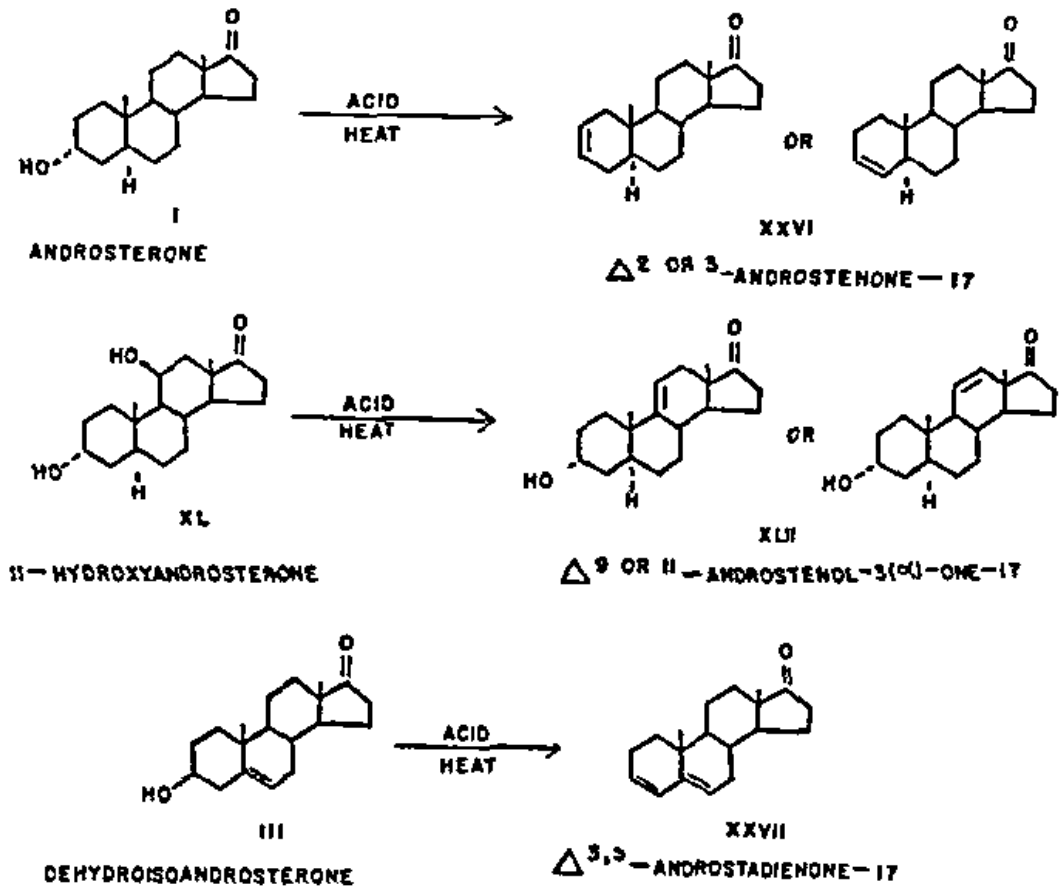


FIG 14 —Artifacts of dehydration

IV Assay of Androgens and Related Substances

The assay of androgens and related substances involves the consideration, first, of the bioassay methods which are dependent upon the biological activity of this class of compounds, and second, of the chemical methods which essentially involve the color produced by C-17 ketones under special conditions Although the biological and chemical methods both measure some of the same compounds, certain obvious differences exist Urinary steroids such as androsterone (I) and dehydroisoandrosterone (III) both possess androgenic activity of varying degrees and give roughly, by the chemical methods usually employed, about the same intensity of color On the other hand, a steroid such as etiocholanol 3(α) one-17 (XVIII) gives a positive test by the chemical methods but is inactive biologically

A Bioassay of Androgens

In studies of extracts containing androgenic material, the biological method is obviously indispensable Biological methods are needed for

the characterization of a new androgen which must include its physiological action (qualitative) and its relative activity (quantitative)

In studies on urinary concentrates it has been shown that a reasonable parallelism exists between the quantity of androgenic material (biological assay) and the amount of 17-ketosteroids (chemical assay) present Oesting (168) has demonstrated that relatively good correlation exists between the androgenic and 17-ketosteroid titer of normal children's urine The work of Callow (31) indicates a close relationship between the two methods in a variety of urines Holtorff and Koch (116) have studied this relationship, and, although their correlation appears to be poorer than that found by the earlier mentioned workers, it appears to be adequate In human urines, for comparative studies either the biological methods or chemical methods may be employed For special studies, the choice of either the biological method, the chemical method, or both must be dependent upon the specific objectives

1 *Capon's Comb Assay by Intramuscular Injection*

The capon's comb has served as a test object for the evaluation of androgenic activity since the time of Berthold s classic experiments It has served not only as a qualitative measure of androgenic activity, but has been used for quantitative studies As a quantitative tool, many variables had to be discovered and controlled *Such factors as* age at which cocks are caponized, breed of capon, weight of capon, influence of light, age of bird, and initial comb size have been subjected to critical analysis Various methods of measuring comb size have been employed such as direct measurement of the comb with a millimeter rule, photograph of the comb with subsequent measurement of the areas, etc

Detailed studies on the capon method have been reported particularly by Gallagher and Koch (91), Greenwood, Blythe, and Callow (96), and McCullagh and Cuyler (146) The method of Gallagher and Koch is representative of the capon method This method consisted in the use of brown leghorn capons Before administration of the test material, the length and height of the comb was obtained by direct measurement with a millimeter rule The capons were injected intramuscularly once daily for five days and the combs again measured one day after the last injection Each daily dose was contained in 1 ml of oil The increase in the length (L) plus the increase in height (H) was taken as the response The response (L plus H) was plotted against dosage and the characteristic curve determined It was found that a response of 3 to 7 mm in L plus H was the desirable range for assays The capon unit was defined as the amount of material which, injected per day for five days, yields an average of 5-mm increase in L plus H This unit is approximately

equal to one international unit The standard preparation employed was a highly purified bull testis preparation in which the activity was due principally to testosterone These workers reported a mean error of 22 6% when the unknown was run in parallel with a standard and groups of 16 to 25 capons were used for both the unknown and standard

Greenwood *et al* (96) have also studied the dose-response relationship using androsterone and the brown leghorn capon The five-day period was employed and the measurement of the comb done in a manner similar to that utilized by Gallagher and Koch A log dose response curve was constructed between the limits of 0 5 and 8 mg of androsterone and was found to give a linear relationship within these limits . The authors found a slope of 12 6 when the comb response (*L* plus *H*) was expressed in millimeters and the dose expressed as logarithm of milligrams The authors claimed an accuracy of $\pm 18\%$ for the determination of an unknown, using five capons, and claimed that if the number of capons employed was increased to ten, the error was decreased to $\pm 12\%$

Although early studies indicated that the initial size of the comb was unimportant (7,89), more detailed studies seemed to indicate that the initial size of the comb must be taken into account for precise assays (91) The weight of the animals makes a slight difference in response No significant difference in response could be attributed to animals varying in age from four months to six years Responses to subcutaneous and intramuscular injections were similar but the amount and nature of the solvent employed was an important factor Variations in intensity of light were reflected in changes in response to a standard dose

A question of the strain of capons suitable for androgenic studies has been investigated It has been found that, in addition to the white and brown leghorn, the English game bantam may be employed However, the heavier breeds such as the Rhode Island Red and the Plymouth Rock are not sensitive enough for the test (171)

2 *Capon Assay by Direct Application to the Comb*

A more sensitive method for the utilization of the capon's comb has been the direct inunction of the androgen on the comb Studies of these methods (50,52,88,146) have indicated that this method is approximately 100 to 200 times as sensitive as subcutaneous and intramuscular injection methods The author is not aware of any statistical studies on the method using capon's comb by inunction, and the accuracy is difficult to evaluate

3 *Chick's Comb Method of Assay*

The early observations of Ruzicka (192), Burrows, Byerly, and Evans (14), Danby (44,45), Dorfman and Greulich (59), and Frank

et al (82,83) indicated the advisability of using the chick comb as the test object for androgen assays Ruzicka painted the chick's comb with a 0 5% solution of androsterone in oil each day for a period of several weeks and obtained large increases in comb area He did not, however, study this reaction quantitatively Frank and Klempner (83) applied the androgens in oil solutions directly to the base of the comb of white leghorn chicks Applications were begun on the sixth day after hatching and were repeated on ten successive days The animals were sacrificed and the comb weights were determined on the day following the last application These workers were able to evoke a definite response with as little as 20 μg of androsterone Burrows and co-workers injected both androsterone and testosterone either into the base of the chick's comb or into the breast muscles and found that both these androgens stimulated comb growth In all the studies mentioned, the end point consists of the weight of the comb, which perhaps represents an advantage over the less exact methods of measurement of size of the capon's comb However, the capon's comb method has the advantage that each animal serves as its own control

The method of Hollander *et al* (115) and Frank *et al* (84) is the most precise of the chick methods suggested and has been demonstrated to be an adequate method for the determination of androsterone and urinary androgen This test was designed to utilize the two-to-three day old white leghorn chick The total dose of material in 0 35 ml of oil was administered in seven divided doses at 24 hour intervals The material was administered by applying the test solution from a hypodermic needle moving lightly over the surface of the comb Twenty four hours after the last administration the animals were killed with chloroform and the combs removed and weighed Mixed male and female chicks were employed The calculations take into consideration the initial and final body weights as well as the sex of the animal and weight of the comb

The following formulation was developed to calculate the androsterone equivalent in terms of milligrams

$$A = \frac{1\,06(\Sigma w) - 0\,0043(\Sigma w^2) - 0\,397(\Sigma B_i) - 0\,267(\Sigma B_t) + 14\,75N_m + 18\,54N_f}{N_m + N_f}$$

where A = androsterone equivalent in mg , Σw = sum of comb weights in mg , Σw^2 = sum of squared comb weights, ΣB_i = sum of initial body weights in g , ΣB_t = sum of terminal body weights in g N_m = number of males, and N_f = number of females

Using this formulation, Klempner (120) has shown that in 24 determinations of androsterone using sixteen animals in a determination in

the dosage range of 20–40 μg , the mean error was 13%, and in 39 determinations over the range of 10–50 μg , the mean error was 24 6% In another study, the results of Klempner were essentially confirmed (58) In the latter study, with the range 20–40 μg of androsterone, a mean error of 12% was found, and in the range 10–40 μg , a mean error of 24% was found

The details of this method have been extended to the assay of testosterone propionate by Dorfman (58) Here the calculations were based on a simultaneous standard run according to the design formulated by Bliss (6) With the use of 32 chicks on the standard and 32 chicks on the unknown, errors in potency ratios of less than ±38% were realized

Increase in light tends to increase the sensitivity of the comb to androgens, at least in the range of complete darkness to normal light (202) The body weights of animals in normal light were higher than those kept in darkness The effect was still preserved, however, if the results were expressed as ratios of comb to body weight

The sensitivity of the chick's comb to androgens varies with the breed employed This is probably true for endogenous androgens as well as exogenous material since the comb ratios (comb weight per unit body weight) vary with the various breeds Thus, when the comb ratios of the White Leghorn, Rhode Island Red, and Barred Rock untreated male chicks are compared, it is found that the White Leghorn is the largest followed in order by the Rhode Island Red and Barred Rock The relative magnitudes of the ratios may be expressed as 8 6 4, respectively When relatively small doses of androgens were administered to male chicks of the three breeds, it was found that when the chick comb ratios of White Leghorns increased 300%, comb ratios of Rhode Island Reds increased 100% and ratios of Barred Rocks increased 70% (58)

4 *Mammalian Assay Methods*

Various mammalian tests, usually on rodents, have been employed for the assay of androgens, such as the weight or histological change of the seminal vesicles or the prostate, the electrical ejaculation test (3,156), the ductus deferens test (210), the Cowper s gland test (104,105), and a pharmacological (pernoston and yohimbine) ejaculation test (127)

Among the various mammalian tests employed, the most important from the standpoint of sensitivity and accuracy has been the weight of the seminal vesicles prostate, or both The studies of Korenchevsky and Dennison (123), Deanesley and Parkes (49), Miescher, Wettstein, and Tschopp (149), Callow and Deanesley (32), Bulbring and Burns (10), and Greene and Burrill (93,95) are important in the development of

these methods Recently Hays and Mathieson (100) and Mathieson and Hays (145) have reinvestigated the use of the seminal vesicles of the castrated rat for the assay of androgens These workers specifically used testosterone propionate and the experimental design of Bliss (6) The assay is so designed that a comparison could be made between standard and unknown solutions of testosterone propionate at two dose levels By this method, an accuracy of $\pm 20\%$ could be achieved if each of the four groups of animals contained eight animals or a total of 32 animals on the standard plus the unknown

In addition to the usual variables which influence biological assay methods—such as weight, strain, and age of animals, volume and nature of solvent (49), and diet of animals—the presence of contaminating estrogens may be considered in the seminal vesicle and prostate tests, since these substances have been shown to have an enhancing action A second factor is the question of such activators as palmitic acid, which apparently exert an enhancing action on the absorption of androgenic substances

5 *The International Androgen Standard*

As a result of the League of Nations Committee meeting held in 1935, an international standard for androgens was established The committee adopted 0 1 mg of androsterone as equivalent to one international unit

B Polarographic Determination of Androgens and Related Compounds

Studies on the applicability of the polarographic method for the determination of androgens and related steroids have been reported by Wolfe, Hershberg and Fieser (219) From the work of these investigators, it appears that the 17-ketosteroids present in neutral urinary extracts can be determined accurately and rapidly by reacting these steroids with Girard's reagent T (trimethylacethydrazide ammonium chloride) and polarographic analysis of a suitable aqueous solution of the reaction mixture Under the conditions of analysis, 3 ketosteroids are indifferent, and the 20 ketosteroids give a distinctly different result than the 17-ketosteroids The Δ^4-3 ketosteroid may be easily distinguished from the 17 ketosteroids

In a preliminary study of the relationship between 17-ketosteroid concentrations in urinary extracts, good agreement was found between the values obtained by the polarographic and Zimmerman methods, although the range was from 1 7 mg to 141 mg of 17 ketosteroids per liter of urine

C Chemical Determination of Androgens and Related Compounds

For specific purposes such as the determination of urinary androgens and their related compounds, many of which are metabolites of body androgens, the chemical methods of detection have been applied as an alternative to biological assay Zimmerman (224,225) demonstrated that pure ketonic steroids such as androsterone, testosterone, and estrone could be quantitatively determined by the use of the reaction of these substances with *m* dinitrobenzene in alkaline solution to produce a characteristic color This work was followed by that of Wu and Chou (223), who modified the test and studied concentrations of color-producing material in urine, and expressed the results in terms of androsterone Following these initial efforts, an extensive literature has appeared dealing with modifications of the method as well as extensive applications to the study of urinary concentrations in normal and abnormal individuals

Although numerous methods for the determination of 17-ketosteroids have been suggested, analysis of some of the factors operating in two of these methods (the details of which show differences) may suffice for our purposes These two representative methods are those of Callow *et al* (28) and of Holtorff and Koch (116)

The method of Callow *et al* (28) consists essentially in dissolving the material to be tested in absolute alcohol, adding a 2% solution of *m*-dinitrobenzene in absolute alcohol, and finally a 2 5 *N* solution of potassium hydroxide in absolute alcohol The solutions are mixed and incubated for one hour at 25 ± 0 1°C and protected from strong light A calibration curve is constructed with known amounts of a crystalline standard such as androsterone The "blank" consists of the solvent, absolute alcohol, plus the *m* dinitrobenzene and potassium hydroxide solutions

The spectroscopic studies of the reaction product between androsterone and *m* dinitrobenzene showed a maximum at 5010 A, while the reagents alone gave a low general absorption with a maximum at 4650 A Callow suggested that any selective filter having maximum transmission somewhere between 5000 and 5400 A would be suitable It was noted that a broad absorption band with a maximum in the green was characteristic of carbonyl substitution at C-17 By this technique, distant substituents had little influence on the spectral characteristics of the color Thus, dehydroisoandrosterone and estrone gave calibration curves similar to that of androsterone Saturated 3 ketones show a very low general absorption after a one-hour development preceded by a rapid color development at five minutes In the case of Δ^4-stenones a longer time is required for the color development, the maximum is not

obtained at one hour This group of compounds also shows a maximum in the yellow in addition to that found in the green The 20 keto group has been shown to give only a low general absorption

The studies on urinary extracts showed that both normal male and female urinary extracts had an absorption spectrum quite similar to that found for androsterone However, in certain abnormal urines the reading in the green was partly due to substances other than 17 ketosteroids In such cases, it is found that relatively high absorptions were found in the region of the violet

In the original work of Callow a good correlation was found between the chemical tests and the androgenic assay In spite of the relatively high error of estimate in the biological assay, a correlation coefficient of 0 745 was found

The question of nonspecific chromogen determined on the total neutral fraction has been studied by a number of workers Essentially two methods have been employed, the first being to perform the determination on ketonic fraction, the second the use of a correction factor

Talbot, Butler, and MacLachlan (206) have shown that higher accuracy can be attained with the Callow method when ketonic fractions are employed Frazier *et al* (85) have used a correction equation to compensate for the overestimates inherent in measurements on the total neutral fraction The interfering chromogens appear to absorb maximally in the region of the violet at 4100 A , as contrasted with the maximal absorption of the 17 ketosteroids at 5200 A The validity of using a correction equation for the Callow procedure has been shown by the fact that net values so obtained agree well with the value derived from assays on the ketonic fractions (76,208)

Applying the formulations of Gibson and Evans (92) and making readings in the green and violet, the following correction equation may be used for the 17-ketosteroid determination by the Callow procedure

$$\text{Corrected reading in green} = \frac{K_t E_G - E_T}{K_t - K_s}$$

For chromogens $K_c = E_v/E_G$

For 17 ketosteroids $K_s = E_v/E_G$

The Holtorff-Koch technique differs from the Callow method in a number of details This method consists in the use of an aqueous 5 *N* potassium hydroxide solution and 95% ethanol solutions of the test material and a 2% solution of *m*-dinitrobenzene in 95% ethanol The time of incubation was originally set at 45 minutes, but subsequent studies have indicated that the maximum color development is obtained at about 105 minutes (164) Unlike the Callow method, this method shows a difference in color produced by various 17 ketosteroids This method shows a departure from linearity as the amount of total urinary

extract employed is increased This is minimized if the measurements are made in the dilute range and completely removed if assays are done on the ketonic fraction even in an extended range Since the curve departs from linearity, correction equations cannot be applied over an extended range of urinary concentrations (76)

Pincus (179) has described a colorimetric method for the determination of urinary 17 ketosteroids which excludes a number of chromogens that react with *m* dinitrobenzene It involves reaction of neutral ketonic steroids with concentrated antimony chloride ($SbCl_3$) in acid solution Androsterone and its isomers produce an intense blue color, whereas the 20 ketosteroids and the 3 ketosteroids give yellowish or colorless reaction products Androstenone-17 reacts as intensely as androsterone, and dehydroisoandrosterone with about one-seventh the intensity of androsterone This reaction is applicable to human urine extracts and has also been used by Cohen and Salter (197) and Venning (211), who find it more specific than the *m* dinitrobenzene reaction (see also Pincus, 179)

With the Holtorff-Koch technique such androgens or 17-ketosteroids as dehydroisoandrosterone and $\Delta^{2 \text{ or } 3}$-androstenone-17 tend to give higher color values than androsterone Therefore, if urines are studied by this method after extraction procedures which cause extensive conversion of androsterone to the $\Delta^{2 \text{ or } 3}$-androstenone-17, the absolute values for 17 ketosteroids tend to be high when androsterone is used as the standard

V. Concentration of Androgens and 17-Ketosteroids in Urine and Blood

During the past fifteen years a rather large body of data has been accumulated with respect to the urinary levels of androgens and 17 ketosteroids in the urine of normal and diseased patients Due to the difficulties in running androgen assays, only a relatively small amount of data on androgen concentrations has been presented, but with the advent of colorimetric methods for the determination of 17-ketosteroids many studies on these constituents of urine were presented, until at present a rather large literature has grown up

Certain dynamic changes in urinary 17 ketosteroids have been found in various conditions of stress The 17 ketosteroid concentrations in urines may be considered at two different levels The first level, which is discussed under the adrenal cortical hormones, is concerned with the adaptation of adrenal cortex to stress, probably by way of pituitary stimulation and may involve changes from hour to hour (see Chapter XIII) The second level deals with the average value of 17-ketosteroid excretion over a period of a day or many days It is the latter level

which concerns us here and which may be correlated with urinary androgen excretion

In discussing levels of 17-ketosteroid excretion in various urines, it is apparent from previous discussions that the magnitude is dependent

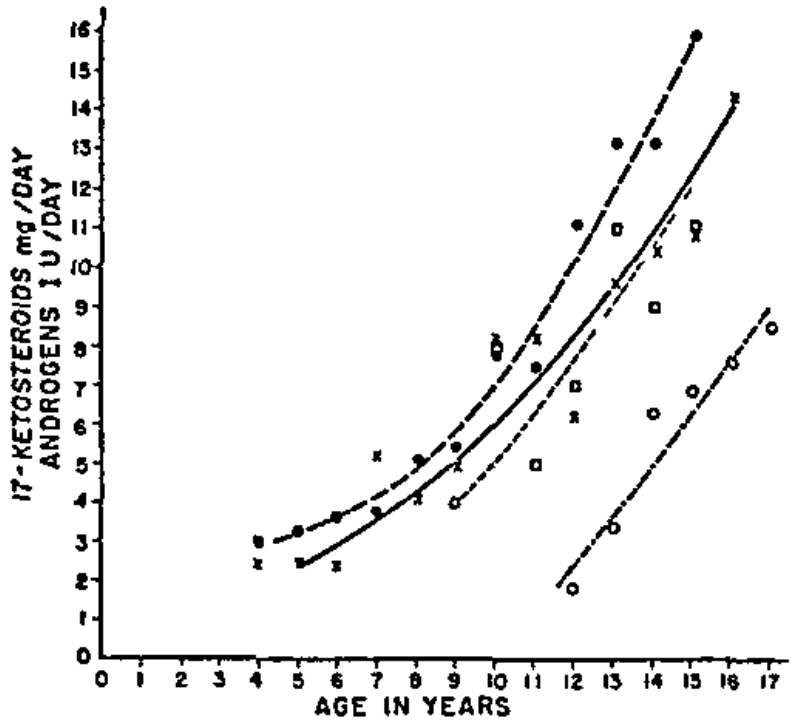


FIG 15 —The excretion of androgens and 17-ketosteroids in the urine of boys.

Boys X = #403 (ref 169)
O = #370 (ref 155)
O = #390 (ref 163)
} 17-ketosteroids
□ = ref 97, androgens

upon such factors as nonspecific substances, the original method of urine extraction, and finally, the method of 17-ketosteroid determination

A CONCENTRATION OF ANDROGENS AND 17-KETOSTEROIDS IN HUMAN URINE

In Tables II-XXII an attempt has been made to compare the values of androgens and 17-ketosteroids reported by different investigators in normal and diseased subjects of varying ages

1 *Children*

During the first six years of life, boys showed an extremely low level of androgenic material in the urine A rather rapid increase in urinary excretion was observed beginning at about six to seven years of age The rate of increase in urinary excretions continues up to about the seventeenth to eighteenth year of life Although levels up to 25 international

units per day are obtained at this time, this concentration appears to be significantly lower than the level found during the twenties and thirties Thus in one study, values of 66, 80, and 75 I U per day were found for young men aged, 20, 21, and 22, respectively, as compared with levels of 25 I U at ages of 17 to 18 (62,97)

TABLE II

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF BOYS

Age, yr	Androgens, I U /day	17 Ketosteroids, mg /day		
	Ref No 97	Ref No 169	Ref No 155	Ref No 163
4–8[a]	<0 3 (2)[b]			
2–3	<1 0 (1)			
3–4	<1 0 (1), 1 3 (1)	2 4 (4)		3 0 (4)
4–5		2 4 (4)		3 3 (5)
5–6		2 4 (3)		3 6 (4)
6–7		5 2 (4)		3 7 (9)
7–8	<1 (1)	4 1 (3)		5 1 (6)
8–9	4 (4)	4 9 (4)		5 3 (8)
9–10	8 (2)	8 2 (2)		7 8 (2)
10–11	5 (5)	8 2 (3)		7 5 (3)
11–12	7 (16)	6 2 (2)	1 8 (9)	11 1 (4)
12–13	11 (11)	9 6 (1)	3 4 (6)	13 2 (3)
13–14	9 (32)	10 4 (6)	6 3 (11)	13 2 (2)
14–15	11 (30)	10 8 (4)	6 9 (9)	15 9 (5)
15–16	22 (32)	14 4 (1)	7 6 (8)	
16–17	25 (23)		8 5 (7)	
17–18	25 (3)			

[a] Days

[b] Numbers in parentheses indicate number of subjects

The increasing concentration of 17 ketosteroids with increasing age appears to parallel the curve found for the androgens In two studies, those of Oesting and Webster (169) and of Nathanson *et al* (163) this was observed (Fig 15 and Table II) Both of these studies deal with 17-ketosteroid determinations on the total neutral fractions of urines without correction for interfering substances In Mason's studies (155) the determinations were run on the total fractions, but his figures have been corrected Here again we have a similar type of curve but the absolute levels of 17 ketosteroids are lower than these found in the later studies

The increasing concentration of androgens and 17 ketosteroids with increasing age in girls presents a picture similar to that found in boys

The data are represented in Fig 16 and Table III The increase in excretion of both androgens and 17-ketosteroids with increasing age is represented by parallel curves The rate of increase of urinary androgens and 17-ketosteroid concentration is practically zero up to ages four to five, when the androgens and 17-ketosteroids rise dramatically The curves of the various workers appear to be in good agreement as to rates of increment although the absolute values differ

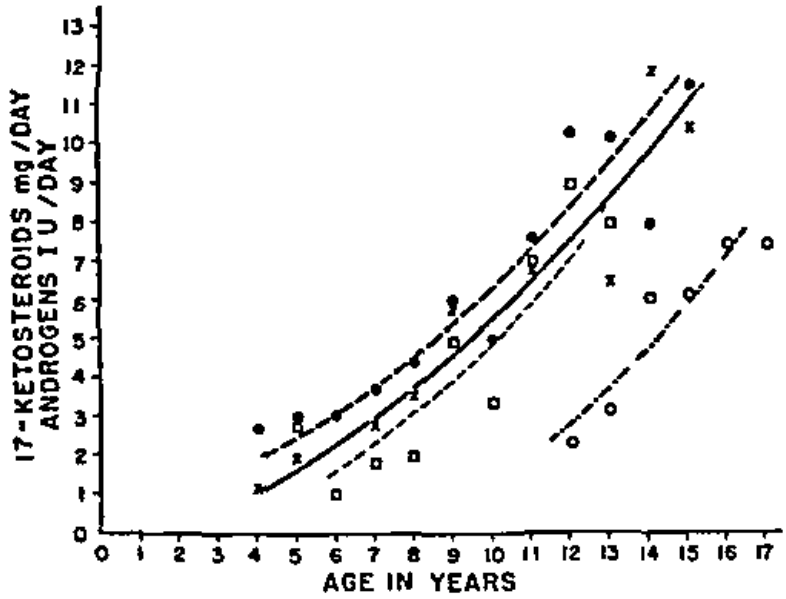


FIG 16 —The excretion of androgens and 17 ketosteroids in the urine of girls

Girls X = #403 (ref 169)
O = #370 (ref 155)
O = #339 (ref 163)
} 17 ketosteroids
□ = ref 58 androgens

It is of some interest that, whether considered on the basis of androgens or 17-ketosteroids, the urinary levels attained by both boys and girls are quite similar In Fig 17, where the data are plotted as age in years versus logarithm of androgen concentration, straight-line functions result for both the boys and the girls, which do not appear to be dissimilar How is one to explain this similarity in rate of increment? It may be argued that in boys the increased urinary androgen concentration mirrors increased production of androgens by the testis But what about the rate of increment of androgens in girls? If we were to attribute androgens of females to the adrenal cortex, we would be forced to the conclusion that the androgen production of the female adrenal gland is greater than that of the male adrenal gland However, another possibility exists—that the ovary does in fact contribute androgens, and further, that perhaps no sexual difference with respect to androgen production of the adrenal exists

TABLE III

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF GIRLS

Age, yr	Androgens, I U /day	17-Ketosteroids, mg /day		
	Ref No 58	Ref No 169	Ref No 155	Ref No 163
3–4	<0 3 (2)	1 2 (1)		2 7 (6)
4–5	2 8 (2)	2 0 (2)		3 0 (5)
5–6	1 0 (2)			3 0 (4)
6–7	1 8 (2)	2 9 (1)		3 7 (5)
7–8	2 (3)	3 6 (2)		4 4 (14)
8–9	5 (2)	5 8 (1)		6 0 (4)
9–10	3 4 (2)			5 0 (4)
10–11	7 (7)	6 8 (1)		7 6 (5)
11–12	9 (4)		2 3 (7)	10 3 (6)
12–13	8 (6)	6 5 (3)	3 2 (9)	10 2 (5)
13–14	18 (5)	12 0 (1)	6 1 (9)	8 0 (3)
14–15	15 (1)	10 5 (4)	6 2 (7)	11 6 (3)
15–16			7 5 (10)	
16–17			7 5 (6)	

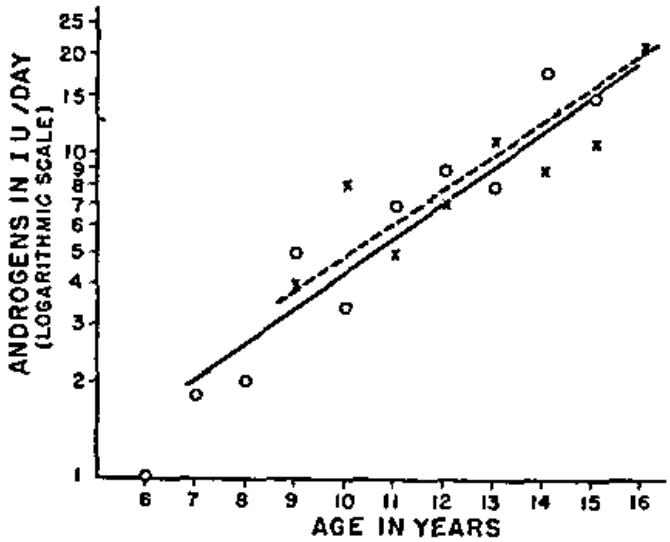


FIG 17 —The excretion of androgens in the urine of boys and girls

○ — girls, X — boys (refs 58, 97)

2 *Normal Adult Men and Women*

The concentrations of androgens in men's urine have been studied by a number of groups of workers utilizing different methods of extraction and assay These data are summarized in Table IV, where it is seen that variations of from three- to tenfold have been observed on the basis of the relationship of the lowest to the highest values found Certain

variations in mean values from laboratory to laboratory are explained on the basis of completeness of extraction and extent of destruction. It may be well to recall that when androsterone (I), the principal androgen in urine, is converted to $\Delta^{3\alpha,5}$-androstenone-17 (XXVI) a tenfold loss of androgenic activity results. The mean values of 38, 66, and 99 I.U./day are quite representative, and there is some reason to believe that the over-all mean of 68 I U /day is reasonable, remembering, however, that the normal values may range down to as low as 20 and up to about 115 I.U.

TABLE IV

EXCRETION OF ANDROGENS AND 17-KETOSTEROIDS IN URINE OF NORMAL MEN

No of subjects	17-Ketosteroids			Androgens			Ref
	Method[a]	Range, mg /day	Mean, mg /day	No of subjects	Range, I U /day	Mean, I U /day	
					19–48		122a
				4 (26–35)	22–115	66	91a
				7 (20–34)	20–225	99	53a
	C		9 1				28a
13	C	9 8–20 8	14 2				76
9	C	8 1–22 6	13 8				85
5	C		13 8				207b
				20 (23–37)	16–86	38	98a
10 (20–40)[b]	C.	11 6–17 5	14 3				199a
11 (17–29)	C.	10 5–19 0	14 1				3a
14 (20–36)	H K	15 9–34 0	22 6				71a

[a] C. = Callow method, H K = Holtorff-Koch Method

[b] Age range

The 17-ketosteroid excretion of normal men appears to range from approximately 8 to 32 mg /day, with a mean in the range of 13 to 14 mg /day when the Callow method is employed With the Holtorff-Koch method and the use of simultaneous hydrolysis and extraction, a range of 15 9 to 34 0 mg and a mean of 22 6 mg has been found on the total neutral extract

Studies on excretion of androgens and 17-ketosteroids in the urine of normal women are presented in Table V, without regard to time of the menstrual cycle since no significant correlations have been reported. The range for androgens appears to be about 20 to 118 I.U./day, although some earlier workers have reported extremely low values, down to 2 to 7 I U /day The mean values appear to be about 40 to 47 I.U./day, or

approximately two-thirds of the amount reported for normal men's urine

TABLE V

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF NORMAL WOMEN

No of subjects	17 Ketosteroids			Androgens			Ref
	Method[a]	Range mg /day	Mean, mg /day	No of subjects	Range, I U /day	Mean, I U /day	
5	C	5 4 to 19 6	12 2				217a
			6 8				28a
15 (20–40)	C	5 8 to 17 0	10 2				76
14	C	5 1 to 14 2	9 0				85
				8 (20–26)	20–68	40 6	53a
				4 (23–34)	22–85	47	91a
					2–50		29a
					7–35		201a
9	C		9 1				207b
5 (21–43)	Os	3 to 28	12 6				98b
3	Os	4 3 to 17 3	9 5				85a
4 (26–38)		3 8 to 4 8	4 5				3a
30 (20–40)	H K	4 0 to 22 0	12 6				71a

[a] Os = Oesting, for other abbreviations, see Table IV

Numerous studies of 17-ketosteroids in the urine of normal women have been reported In studies utilizing the Callow technique, mean values from 6 8 to as high as 12 2 mg have been reported, with the mean somewhere around 9 4 mg /day, or, as in the case of androgens, about two thirds the values found for normal men In one instance a mean value of 4 5 mg /day was reported, but there is evidence that in these studies some of the 17-ketosteroids were lost before the color reaction was employed The Holtorff-Koch method on some thirty subjects between the ages of twenty and forty years of age has yielded a range from 4 0 to 22 0 mg with a mean value of 12 6 mg /day The most variable data presented are a study on some five women in which a range of 3 to 28 mg /day was found with a mean of 12 6 mg /day This latter study utilized the Oesting method

3 *Senile Men and Women*

From a few reports on the concentrations of androgens and 17-ketosteroids in the urine of old men, it is apparent that in old age there is a decrease in these urinary constituents (Table VI) Thus, while one laboratory reports a mean value of androgens of 99 I U /day for men 20 to 34 years of age, they found a value of 20 I U /day in a group of six

men 59 to 67 years (Table VI) In another study men of the age group 50 to 76 years were reported to excrete only 10% of the amounts of androgens excreted by young men

TABLE VI

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF OLD MEN

No of subjects	17 Ketosteroids			Androgens			Ref
	Method*	Range mg /day	Mean mg /day	No of subjects	Range I U /day	Mean I U /day	
				(50–76)	2–3		122a
				1 (79)		<5	53a
				1 (87)	10–25	18	53a
				6 (59–67)	5–40	20	53a
5 (71 75)	C	2 8–4 3	3 4				85
4 (82 88)	H K	2 9–12 0	5 9				71a
1 (62)	H K	6 3–12 5	9 4				71a

* For abbreviations see Table IV

In two studies on 17 ketosteroids in older men it was shown by the Callow method that men 71 to 75 years of age excreted about one third that excreted by normal men while in the second study utilizing the Holtorff Koch method four men 82 to 88 years of age excreted one fourth the amount found in the urine of men 20 to 36 years of age (Table VI)

TABLE VII

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF OLD WOMEN

Age	Years since last menses	17 Ketosteroids mg /day	Androgens I U /day	Ref
65–72	?		<9–11	53a
49	0 42	53 (13)*		98a
48	0 75	38 (20)		98a
42	0 75	55 (11)		98a
47	1	38 (26)		98a
52	2	81 (12)		98a
57	2	59 (4)		98a
48	2	60 (7)		98a
56	4	38 (8)		98a
56	5	38 (7)		98a
62	14	32 (15)		98a
67	25	16 (4)		98a
74	26	22 (24)		98a

* Number of determinations is shown in parentheses

The excretion of androgens by old women appears to be lowered Thus women 65 to 72 years of age were found to excrete approximately one fourth the amount of androgens excreted by normal women of 20 to 26 years However, dramatic increases in 17 ketosteroid excretion

TABLE VIII

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF EUNUCHOID MEN

Age yr	17 Ketosteroids, mg /day	Androgens, I U /day	Ref
18	8 5		85
18		27	99a
19	2 8		85
20	2 4		85
20		9	145a
20		17	119a
21		15	145a
21	4 8		85
21	6 8		85
22		20 5	99a
23		23 0	119a
23		4	145a
24		17	119a
24	3 5		85
25	8 4		85
26		28	119a
26		9	99a
27		24	99a
27		8	119a
28		14	99a
28	4 2		85
29	6 4		85
29		13	119a
29		15	119a
30		6 7	119a
30		23	145a
31		1	119a
31		21	119a
33		6	145a
34	3 9		85
34		1	145a
35	3 0		85
35		14	99a
36		33	119a
39	13 3	65	29a
42		23	99a
44	13 1	80	29a
56	9 4	8	29a

have been reported by one group of workers (Table VII). These observations have not been confirmed

TABLE IX

EXCRETION OF ANDROGENS AND 17-KETOSTEROIDS IN URINE OF CASTRATE MEN

Age, yr.	Years since castration	17-Ketosteroids, mg /day	Androgens, I U /day	Ref.
38	0 04		2 7–5 5	99b
26	0 08		5 1*	99b
66	0 1	5 4		85
29	0 17		6 9*	99b
27	0 5		4 1*	99b
29	0 5		17 5*	99b
Adult	0 5		5 7*	99b
47	1		15 4*	99b
24	1		28*	29a
38	2		>15*	99b
44	2		14	98a
52	2		0	98a
21	2		1 66	119a
41	2 5		3 4*	99b
36	4 5		> 4 5*	99b
19	5 0		18	29a
48	5 0		22*	99b
64	6 0		0	98a
62	6 5		4 7*	99b
56	7		11	98a
58	7		2 9*	199a
47	8	7 0		85
45	10		10	29a
56	13		9	99a
39	13		11	99a
52	14		7	98a
38	19		18	29a
45	20		7	29a
45	21		11	29a
46	22		11	29a
43	22		8	99a
45	26		3	99a
54	33	3 2		85
56	36		5 8	119a

* Values on a liter basis

The decreases in androgenic material in old age, which appears to be of the order of at least 75%, may well indicate both a decreased function of the gonads and of the adrenal cortex

4 *Eunuchoid and Castrate Men*

Table VIII is a summary of urinary assays on eunuchoid men ranging in age from 18 to 56 years The data are arranged according to chronological age A relatively large variation in androgens and 17-ketosteroids is apparent Thus androgen levels from as low as 1 I U /day to 80 I U /day have been reported, and 17 ketosteroid levels of from 3 to more than 13 mg /day In general the values for androgenic material are lower than normal With respect to 17-ketosteroids, the levels in eunuchoid men are usually less than one-half the amount excreted by normal men

Castrated men still excrete androgens, but the amounts are lower than those for normal men and eunuchoid men (Table IX) The few values reported for 17 ketosteroids in urine of castrated men indicate amounts of the order of that found for eunuchoid men The mean androgenic excretion by castrated men is of the order of 10 I U /day, or one sixth that of normal men No apparent correlation has been observed between the titer of androgens in the urine and the time since castration

5 *Hypogonadal and Ovariectomized Women*

In eunuchoid women, a decrease of approximately one third in 17-ketosteroid values has been observed (Table X) No pertinent data on androgen excretion have been reported

TABLE X

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF EUNUCHOID WOMEN

Age yr	17 Ketosteroids mg /day (Ref 85)
16	3 5
18	4 7
18	4 8
18	7 2
20	4 7
21	2 2
21	2 0
24	8 1
25	6 0
27	14 3
29	8 3
29	4 2
32	4 4
32	9 2
33	5 0
35	11 4

Studies on ovariectomized women present a rather complex story The 17 ketosteroids in the urine of ovariectomized women as determined

by Callow indicate no change, when all the data are considered, from 0 03 to 4 0 years after operation During the same period, however, the androgens appear to have been decreased to one half the normal values No striking difference appears to have been found between the period immediately following operation and, after a number of years, for either the androgens or 17-ketosteroids

TABLE XI

EXCRETION OF ANDROGENS AND 17-KETOSTEROIDS IN OVARIECTOMIZED WOMEN

Years since operation	Age	17-Ketosteroids mg /day	Androgens I U /day	Ref
0 03 (9 days)	30	9 0		98a
0 03 (11 days)	35	7 8		98a
0 034 (12 days)	37	14 4		98a
0 034 (12 days)	41	21 0		98a
0 04	45	A, 5 9, B, 11 1	A, 10, B, 22	29a
0 04	38	A, 10 6, B, 8 6	A, 5, B, 36	29a
0 06	53	5 4	1	29a
0 08	34		13	29a
0 12	27	6 5	17	29a
0 16	38	9 0	3	29a
0 25	22	6 8	2	29a
0 33	32[a]		17	29a
0 58	34	34		98a
0 75	19	6 2	24	29a
1 00	43		14	29a
1 00	56		1	29a
1 00	33	11 8		85
1 00	30	11 9		85
1 00	45	7 0		85
1 33	33[a]	10 0	59	29a
1 50	33[a]	7 4	6	29a
1 5	54		4 5	29a
1 67	45	4 9		85
2 0	32	5 0	2 5	29a
2 0	27	9 2		85
3 0	32	33		98a
4 0	53	5 5	12	29a
4 0	23[b]	19 4	135	29a
4 5	23[b]	21 4	63	29a
5	23	53		98a
7	42	60		98a
8	21	43		98a
16	45	25		98a
16	48	23		98a

[a] Same patient

[b] Same patient

On the other hand, studies by Hamblen's group indicate great increases in 17 ketosteroids following ovariectomy Some time after ovariectomy, these workers reported increases up to 300 and 500% These data are tabulated in Table XI

6 *Addison's Disease*

In Addison's disease, in both male and female patients, androgens and 17-ketosteroids are decreased In male patients, the excretion of 17-ketosteroids is approximately one third that of normal individuals whereas the androgens appear to be approximately 50% of the normal values (Table XII)

TABLE XII

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF ADDISON S DISEASE PATIENTS

Age range	Sex	No of patients	17 Ketosteroids, mg /day	Androgens I U /day	Ref
21–34	M	9	8 5 (1 3 to 12 0)		71a
18–38	M	3		26 (20 to 30)	98a
	M	3	4 9 (3 5 to 7 5)	16 (7 to 23)	28a
33–58	M	3	2 7 (2 1 to 3 5)		85
23–64	F	5	<0 5		85
	F	4	5 0 (2 7 to 7 5)	18 (10 5 to 26)	28a
30–38	F	2	3 1 (1 3 to 4 4)		71a

In women, Addison's disease appears to decrease the androgenic titer approximately 70% while the concentration of 17-ketosteroids is decreased to approximately one third However, one group of workers (85) claims that in women with Addison's disease no 17 ketosteroids are excreted (Table XII)

7 *Pituitary Abnormalities*

In pituitary insufficiency, extremely low values for 17 ketosteroids have been found for both male and female Urinary androgens have been studied in only one patient with pituitary insufficiency and in this patient the values were extremely low (Table XIII)

The quantity of 17-ketosteroids in the urine in acromegaly appears to be highly variable Of the six patients reported, two showed low values while two showed slightly increased amounts The single male patient had a low normal value (Table XIV)

In women with Cushing's syndrome (basophilism) without demonstrable tumor of the adrenal cortex, there appears to be a significant

increase in 17-ketosteroids with a tendency toward a decrease in androgens The urines of only two patients have been assayed for androgens (Table XV)

TABLE XIII

EXCRETION OF ANDROGENS AND 17-KETOSTEROIDS IN PITUITARY INSUFFICIENCY

No. of patients	Sex	Age	17-Ketosteroids, mg /day	Androgens, I U /day	Ref
1	M	42	2 6	0 8	28a
1	M	18	0 6		76
1	M	30	1 6		143
7	M	14–55	<0 5 to 1 8		85
7	F	20–45	<0 5		85

TABLE XIV

EXCRETION OF 17-KETOSTEROIDS IN ACROMEGALY

Sex	Age	17-Ketosteroids, mg /day	Ref
F	26	15 1	217
F	28	4 5	85
F	30	20 4	217
F	35	10 2	85
F	41	3 6	85
F	43	10 6	85
M	31	8 3	85

TABLE XV

EXCRETION OF ANDROGENS AND 17-KETOSTEROIDS IN CUSHING'S SYNDROME (BASOPHILISM), WITHOUT TUMORS OF ADRENAL CORTEX

Sex	No of subjects	Age	17-Ketosteroids, mg /day	Androgens I U /day	Ref
F	2	26–43	13 (11 2 to 14 8)		207b
F	1	12	15	14	41a
F	1	33		6	41a
F	17	16–36	20 9 (9 3 to 60)		217
F	3	26–49	13 9 (10 4 to 19 7)		85

8 *Thyroid Disease*

Hyperthyroidism appears to cause a decrease in androgen and 17-ketosteroid excretion in both men and women (Table XVI). From the available literature, there appears to be a greater percentage decrease

in the female than in the male. A similar situation was found with respect to myxedema, but the percentage decreases for both men and women were proportionately greater. Thus for women suffering from myxedema, a decrease of as much as 84% has been observed

TABLE XVI

EXCRETION OF ANDROGENS AND 17-KETOSTEROIDS IN THYROID DISEASE

Diagnosis	No. of patients	Sex	Age	17-Ketosteroids, mg /day	Androgens, I U /day	Ref
Hyperthyroidism	3	F	20–38	3 6	2	29a
	11	F	25–67	7 2		76
	5	F	28–74	2 8		85
	2	F	20	7.7	2	3a
	2	M	38–40	8 6	59, > 44	29a
	11	M	24–63	10 4		76
	6	M	32–51	8 8	6.4	3a
Myxedema	7	F	33–67	1 8	.	76
	6	F	14–63	1 3	.	85
	2	M	39–51	4 3	.	76

9 *Testicular and Embryonic Tumors*

A survey of the concentrations of 17-ketosteroids in the urines of individuals suffering from various testicular and embryonic tumors is presented in Table XVII. In one case of interstitial cell tumor, a value

TABLE XVII

EXCRETION OF 17-KETOSTEROIDS IN INDIVIDUALS WITH VARIOUS TESTICULAR AND EMBRYONIC TUMORS

Diagnosis	Age	Sex	No of patients	17-Ketosteroids, mg /day	Ref
Interstitial cell tumor	41	M	1	1015	212
Chorionepithelioma	32	F	2	23	217
	22–24	F	2	8 9	71a
Teratoma testis		M	1	28 6	217
	21–29	M	2	22 9	71a
Macrogenitosoma?	8–15	M	2	11 4	217
Hydatiform mole	26	F	1	7.3	71a
Seminoma		M	4	21 4	217

of 1015 mg /day of 17-ketosteroids was found. In teratoma testis the mean of three cases seemed to indicate a slight increase in 17-ketosteroids Two cases of macrogenitosoma in prepubertal boys indicated a level of 17-ketosteroids equivalent to that found in normal boys of this age

group In four cases of seminoma, there appears to be an increase of 50% in 17-ketosteroids above the levels in normal men

The levels of 17-ketosteroids in women suffering from chorionepithelioma and hydatiform mole appeared to be within the normal range

10 *Precocious Puberty*

Various types of precocious puberty in children are presented in Table XVIII

TABLE XVIII

EXCRETION OF 17-KETOSTEROIDS IN CHILDREN WITH PRECOCIOUS PUBERTY OR VIRILISM

Sex	Age		Remarks	17 Ketosteroids, *mg /day*	Ref
	Yr	Mo			
F	4	0	Breasts and uterus enlarged	8 9	162a
F	4	1	Breasts enlarged	4 0	162a
F	6	6	Penis like clitoris	9 3	217
F	6	8	Menses at 6 yr , 6 mo	6 8	162a
F	7	1	Menses, breasts enlarged	6 0	162a
F	7		Virilism	90	217
F	9		Precocious puberty	30	217
F	9		Menses	8 4	217
F	10	5	Virilism	140	217
F	10	5	Basophilism	21	217
F	11	2	Menses at 10-11 yr	10	162a
F	13		Virilism	13	217
F	13		Precocious puberty	19 8	217
M	4	3	Precocious	3 5	162a
M	11	1	Precocious	15 7	162a

11 *Hirsutism*

Androgenic and 17-ketosteroid assays in hirsute women are listed in Table XIX This rather heterogeneous group of patients have an increased 17-ketosteroid excretion of the order of 100% while androgens appear to be within the normal range

12 *Ovarian Tumors*

The question of concentrations of androgens and 17-ketosteroids in patients with ovarian tumors has not been satisfactorily answered In Table XX some studies are listed in which no increments of 17-ketosteroids were found in a case of arrhenoblastoma and a case of disgerminoma However, one worker (Warren, 1944) reports an "ovarian tumor" patient who excreted 158 mg /day of 17-ketosteroids

TABLE XIX

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN URINE OF HIRSUTE WOMEN WITHOUT KNOWN ADRENAL CORTICAL HYPERACTIVITY

No of patients	Age	Remarks	17-Ketosteroids, mg /day	Androgens, I U /day	Ref
20	19–30[a]	"Adrenal virilism" —no tumor	22 (5 9 to 37)		217
11	20–36	Hirsutism	16 (3 4 to 33)		217
67		Hirsutism	(6 4 to 33 4)		173a
5	20–35	Mild hirsutism of unknown origin	29 (20 to 35)		71a
			(7 5 to 30)		31a
15	17–36	Hirsutism		47 (8–116)	119a

[a] Some with age not designated

TABLE XX

EXCRETION OF ANDROGENS AND 17 KETOSTEROIDS IN WOMEN WITH OVARIAN TUMORS

Diagnosis	Age	17-Ketosteroids mg /day	Ref
Arrhenoblastoma	30	6 9 preop 3 2 postop 5 wk	85
Disgerminoma	19	7 8 preop 2 5 postop	85
"Ovarian tumor"	31	158	217

13 *Adrenal Cortical Hyperactivity*

The excretion of androgens and 17-ketosteroids in the urine of patients with adrenal cortical hyperactivity, which includes hyperplasia and tumors, is listed in Table XXI

The cases are listed according to age, with a range from one year to 63 years Three patients are included for whom no specific age is designated The highest titer of 17-ketosteroids obtained from the urine of a human being was found in the urine of a thirty-year-old woman suffering from adrenal cancer with extensive metastases This urine contained 2100 mg /day of 17-ketosteroids Over 700 mg of dehydroisoandrosterone was isolated in pure form from the urine of this patient (74)

14 *Urine 17-Ketosteroid Excretion in Miscellaneous Conditions*

Bruger, Rosenkrantz, and Lowenstein (8), using the Friedgood and Berman method of 17-ketosteroid determination, found a significantly lower 17-ketosteroid excretion in hypertensive women Thus fourteen

TABLE XXI

EXCRETION OF ANDROGENS AND 17-KETOSTEROIDS IN URINE OF PATIENTS WITH ADRENAL CORTICAL HYPERACTIVITY

Sex	Age	Diagnosis	17-Ketosteroids, mg /day	Androgens, I U /day	Ref
F	1	Adrenal tumor	3 0		77
M	1	Adrenal tumor	3 0		77
M	1	Adrenal tumor	28		173a
M	2	Addison's disease, adrenogenital syndrome	15		207b
F	3	Adrenal tumor	170		77
F	3	Adrenal cancer	400		61a
M	3	Adrenal cancer	34		145b
F	3	Pseudohermaphroditism with adrenal cortical hyperactivity	13 5		77
F	3	Adrenal cancer	160		207b
F	3 5	Adrenal tumor	176		85
M	4	Hyperplasia	18		207a
M	4	Hyperplasia	7 8		207a
F	5	Pseudohermaphroditism with adrenal cortical hyperactivity	37		77
F	5	Hyperplasia	19 2		207a
M	5	Hyperplasia	20 3		207a
M	5	Adrenal tumor	27		173a
F	5 5	Pseudohermaphroditism with adrenal cortical hyperactivity	12 6		85
F	6	Hyperplasia	16 7		207a
F	6	Adrenal cancer	850	2200	41a
M	7	Adrenal cancer	275–420		111
F	7	Hyperplasia	30		207b
F	8	Hyperplasia	17		207b
F	8	Adrenal cancer	83	350	218
M	8	Hyperplasia	29 2		85
F	9	Hyperplasia	25 2		207a
M	9	Hyperplasia	33 4		207a
F	10	Adrenal cancer	325		85a
F	11	Hyperplasia	23 4		207a
F	11	Hyperplasia	29		207b
F	12	Hypertrophy		93 preop	68a
F	13	Hyperplasia	23 4		207a
F	13	Adrenal cancer	133		207a
F	16	Adrenal cancer	11 36		85
F	16	Adrenal tumor	54 6		77
F	17	Adrenal cortical adenoma	160		128a
F	19	Pseudohermaphroditism with adrenal cortical hyperactivity	75 2		77
F	21	Adrenal tumor	240		77
F	23	Pseudohermaphroditism with adrenal cortical hyperactivity	60		207b

TABLE XXI (*Continued*)

Sex	Age	Diagnosis	17 Ketosteroids, mg /day	Androgens, I U /day	Ref
F	23	Enlarged adrenals	72 4		85
F	25	Adrenal cancer	367		3a
F	25	Adrenal tumor	215		2a
F	25	Adrenal cancer	64	145	41a
F	25	Adrenal tumor	131		77
F	28	Adrenal tumor	61		31a
F	30	Adrenal cancer	2100		71a
M	34	Adrenal cancer		50–100	13a
F	34	Adrenal tumor	270		173a
F	38	Adrenal tumor	53		31a
F	40	Adrenal cancer	126		217
F	41	Adrenal tumor	77		31a
F	42	Adrenal cancer	690		217
F	45	Adrenal tumor	840		77
F	46	Adrenal cancer	516		71a
F	54	Adrenal cancer	83		217
F	56	Adrenal cancer and Cushing's disease	74		85
F	61	Adrenal tumor	17 3		31a
F	63	Adrenal tumor	45 6		77
F	Adult	Adrenal tumor	170		173a
F	Adult	Adrenal tumor	47		207a
F	Adult	Adrenal cancer	74		207a

normal females between the ages of 20 and 76 excreted 14 2 ± 4 0 mg of 17 ketosteroids as compared with a value of 8 4 ± 4 3 mg for forty hypertensive women between the ages of 28 and 70 years Warren (217) described a 38-year old woman with cancer of the splenic flexure of the colon, who excreted 80 mg /day of 17 ketosteroids Apparently this was a primary lesion, although no further information is included

In a detailed study (155), it was observed that diabetics of all age groups tend to excrete significantly smaller amounts of 17-ketosteroids than normal individuals However, the authors were unable to find any correlation between the level of urinary 17-ketosteroids and the severity of the disease

The question of androgen and 17-ketosteroid excretion in pregnancy has been studied Hain (98) observed a decreased excretion of androgens in human pregnancy, while Pearlman and Pincus (174) report no significant changes in the titer of urinary 17-ketosteroids during pregnancy

The changes in androgens and 17-ketosteroids have been studied in starvation and anorexia nervosa The urinary androgen concentration

decreased from 100 I U to 40 I U /day in a normal male during a four-day fast, while the 17-ketosteroids in the ketonic fraction decreased from 23 mg /day to 9 6 mg and returned to 24 5 mg Low levels of 17-ketosteroids have been found in anorexia nervosa (85)

TABLE XXII

SUMMARY OF ANDROGENS AND 17 KETOSTEROIDS IN HUMAN URINES

Males			Females		
Status	Per cent of adult male levels		Status	Per cent of adult female levels	
	Androgens	17 Ketosteroids		Androgens	17 Ketosteroids
Boys, 5 yr	3	5	Girls, 5 yr	5	5
10 yr	10	10	10 yr	10	15
14 yr	20	50	14 yr	25	60
Old men	15	30	Old women	25	
Castrated men	40	17			
Eunuchoid	40		Eunuchoid	33	
			Ovariectomized[a]	50	100
Addison's disease	50	38	Addison's disease	70	36
Pituitary insufficiency	3	5-10	Pituitary insufficiency		10
			Cushing's syndrome[b]	40	163
Hyperthyroidism	50	80	Hyperthyroidism	10	60
Myxedema		57	Myxedema		16
Interstitial cell tumor of testis		10,000	Chorionepithelioma		100
Seminoma		150	Hydatiform mole		100
Teratoma testis		133	Hirsutism (without tumor)	100	200
Macrogenitosoma (prepubertal boys)		100			
			Adrenal cancer	Up to 4000	Up to 20 000

[a] Callow

[b] Basophilism, without adrenal tumor

15 *Summary of Changes in Androgens and 17-Ketosteroids in Human Urines*

Table XXII is a summary of the concentrations of androgens and 17-ketosteroids in the urines of normal and diseased human beings The table is presented to illustrate relative magnitudes, rather than to

give a strict range of concentrations of these substances in the various conditions

B Androgens and 17-Ketosteroids in Urines Other than Human

1 *Stallion, Ram, Bull, and Rat Urine*

Although man is unique in the rather high amounts of androgens excreted in his urine, androgens have been detected in the urine of other species Stallion urine has been found to contain 8 I U /l , ram urine 4 I U /l , while bull and rat urine have been shown to contain less than 1 I U /l (121) The urines of two primates other than man have been studied, the chimpanzee and the monkey

The urines of two species have yielded crystalline androgens in low yields Dehydroisoandrosterone has been isolated in yields of the order of 0 01 mg /l of bull and cow urine (136) From the same urines, this investigator also isolated androsterone

2 *Chimpanzee Urine*

Androgens have been studied in the urine of two adult female and four adult male chimpanzees (81) One female excreted between 2 and 4 5 I U /day with a mean of 3 1 I U The second varied from 1 5 to 8 I U with a mean of 3 7 I U The mean value for four normal adult males was 6 8 I U with a variation from 3 to 13 5 I U /day

3 *Monkey Urine*

Rather more complete data have been presented for the concentrations of androgens and 17-ketosteroids in the urine of monkeys Table XXIII is a summary of the androgen and 17-ketosteroid excretion in normal adult male and adult female monkeys, immature monkeys of both sexes, and pregnant monkeys

TABLE XXIII

Excretion of Androgens and 17 Ketosteroids in Urine of Rhesus Monkeys

Sex	Status	No of animals	Androgens, I U /day	17 Ketosteroids, mg /day (ketonic fraction)	Ref
F	Immature	2	<0 05		64,72a
M	Immature	2	<0 05		64 72a
F	Adult	6	2 2		73
F	Adult	4	1 2	1 6	68
M	Adult	3	2 6		72a
M	Adult	5	2 1	2 0	68
F	Pregnant	3	5 9*		73

* Minimum value since 10 of 26 assays were minimum due to lack of extract

In studies on monkeys, Dorfman and Van Wagenen (73) found an increase in androgens during pregnancy Although the estrogen concentrations dropped to the normal levels immediately postpartum, the high levels of androgen excretion persisted for about one month The increased androgen excretion in pregnant animals was of the order of 300%

In a study on the source of androgens and 17-ketosteroids in monkeys, the excretion of these substances was studied in five female and four male intact monkeys and after gonadectomy or adrenalectomy, or after gonadectomy and adrenalectomy After gonadectomy, the urinary excretion of 17-ketosteroids usually (but not invariably) increased during the first postoperative week This was followed by a secondary decrease in 17-ketosteroids either to the preoperative level (in the female) or to a value below the preoperative level (in the male) The excretion of androgens decreased after castration in male monkeys Ovariectomy resulted in a decreased androgen excretion in one female monkey and no change in a second female (68)

Even after the removal of the gonads and adrenals both the male and female monkeys continued to excrete about one third the preoperative level of 17-ketosteroids and androgens Since all the adrenalectomized gonadectomized animals died of adrenal insufficiency and since no adrenal tissue could be detected at autopsy, the possibility exists that there may be an extragonadal extraadrenal source of 17-ketosteroids and androgens (68)

C Concentration of Androgens in Blood

No extended study on the concentrations of androgens or androgen metabolites in blood has been reported However, certain preliminary evaluation may be made on the basis of the reported data Although early reports (15,16) claimed that the oral administration of as little as 5 ml of bull, ram, or stallion blood per day for 10 days caused significant androgenic stimulation in capons, later workers found extracts equivalent to 300 to 600 ml of bull s blood necessary for comb growth in the capon (220) Androgens have been found in human blood by two groups of workers (122,147) In one study, ether extracts of blood were found to give capon comb stimulation at the equivalent of 50 ml of blood In the other study 136 ml of blood equivalent were found necessary

VI. Metabolism of Androgens

A Absorption of Androgens by Various Routes

Although early investigators maintained that androgens were not absorbed from the gastrointestinal tract, it was demonstrated by Dorf-

man and Hamilton (61) that the androgen testosterone was indeed absorbed but that the biological response by this route was approximately one sixth of that found after intramuscular injection In later studies by these workers it was demonstrated that other androgens, such as androsterone, dehydroisoandrosterone, Δ^4 androstenedione-3,17, androstanedione 3,17, and androstanediol-3(α),17(α), may be absorbed from the gastrointestinal tract, as evidenced by considerable excretion of androgenic substances in the urine of individuals receiving these androgens by mouth (66)

In a study designed to compare the rates of absorption of testosterone propionate from the gastrointestinal tract by intramuscular injection and by pellet implantation, it was found that a dose of testosterone propionate administered orally or intramuscularly caused a significant rise in urinary androgens within two hours of the time of administration Intramuscular administration did not result in as great an increase in urinary androgens immediately but resulted in a longer continued excretion of androgens Twenty mg of testosterone propionate in a single intramuscular injection in oil increased the titer of the urinary androgens in a castrate with very low excretion of androgens (3 I U /day) to a titer equivalent to 34 I U /day for about 24 hours Between the thirty-ninth and forty-eighth hours after hormone administration, the urinary androgen level decreased to about 12 I U /day (67)

In the same studies it was found that the implantation of four pellets (280 mg) of testosterone propionate maintained a level of 34 I U /day (value equivalent to that of normal man) for six weeks, in a castrated man whose pretreatment values were approximately 8 I U /day During this six-week period, the patient was free of symptoms

The intramuscular administration of the same amount of testosterone propionate in oil could be expected to produce the same effect for only three weeks Further, it should be mentioned that after eight weeks the patient receiving pellets was excreting androgens at the rate of 16 I U /day, which still represented a 100% increase above the control level The mean surface area of each pellet was 42 mm ^{2}

Deanesley and Parkes (50) have shown that intramuscular or subcutaneously implanted crystalline material was only slowly absorbed, but efficient in effect on the organism Hamilton and Dorfman (99) were able to confirm Deanseley and Parkes in a study comparing the effects of testosterone propionate crystal implantation with injection in various solvents The test object was the chick's comb When 20 mg of androgen was employed, a striking effect was obtained for 71 days with crystals, as compared with lesser effects with beef tallow, palmitic acid, peanut oil spermaceti wax, and mineral oil preparations

Peanut oil, an example of the oils usually employed as solvent in clinical therapy and laboratory experiments, showed much less intense effect than the crystals, and for only seventeen days

In studies on the absorption of pellets of testosterone and its esters, Emmens (75) was able to show a remarkably steady rate instead of the sharp decrease theoretically expected This is explained on the basis of irregularities which develop on the surface of the pellet He was also able to show that pellets of testosterone esters have a decreased rate of absorption as compared with the free compound

Androgens may be absorbed directly through the skin (157) Sublingual administration also appears to be effective A review of these studies has been published by Corner (40) Androgens appear to be one fourth as active by the sublingual route as by intramuscular administration Miescher and Gasche (149) found that sublingual administration of testosterone and methyltestosterone to castrated rats was twenty to thirty times more effective than administration by stomach tube These studies were done with alcoholic solutions

B Anabolic Considerations

The metabolism of androgens may be considered in two categories, namely, the anabolism and the catabolism Further, in these subdivisions we may consider the different androgens according to their origin and structure With respect to the anabolic phase, little is known at the present time Some preliminary work has been done in which various androgens have been perfused through the testes and the resulting change in biological activity has been studied (45) Testosterone added to blood which was perfused through bull's testes caused no increase in androgenic potency When dehydroisoandrosterone (III), Δ^5-androstenedione-3,17, (XXII) or Δ^5 androstenediol-3(β),17(α) (XXVIII) were added to the blood, the perfusate contained increased amounts of androgens Although metabolic changes probably did take place, no decisive conclusions may be drawn from these experiments

Our knowledge of the metabolism is mostly limited to studies of tissue hormones, urinary excretion products and experiments involving administration of various androgens and determination of the urinary excretion products No isolations of blood constituents have thus far been announced

The structure of the urinary steroids must be considered from the standpoint of the methods used for their isolation The methods involving rather drastic conditions such as strong mineral acid and heat, often produce artifacts of dehydration and substitution and may produce artifacts of degradation

C Catabolic Considerations

1 *Metabolism of Testosterone*

The metabolism of testosterone has been studied extensively Beginning with early demonstrations that the administration of testosterone resulted in increased excretion of biologically active material in the urine, this work has led to the isolation and identification of some of the transformation products That testosterone administration either orally or by intramuscular injection gives rise to increased excretion of androgens and 17 ketosteroids in the urine has been shown for men by Dorfman and Hamilton (63), Callow *et al* (29) and Hoskins and Koch (118) This conversion is also true for the rat (56), the guinea pig (60), the monkey (64) and the chimpanzee (80)

The findings of Callow (27) and of Dorfman, Cook, and Hamilton (59)—that at least a portion of the administered testosterone may be accounted for by the conversion to the biologically active androgen, androsterone—showed the relationship between the tissue hormone and a urinary constituent In addition to androsterone, it was also shown that two other isomers result from the metabolism of testosterone, namely, etiocholanol 3(α)-one-17, by Callow (27) and Dorfman and Hamilton (66), and isoandrosterone by Dorfman (57) Androsterone, etiocholanol 3(α) one 17 and androsterone have been isolated from the urine of the chimpanzee after the administration of testosterone by Fish and Dorfman (80) The administration of testosterone to a normal woman has resulted in the excretion of the same metabolites with the possible addition of androstanediol 3(α),17(α) (198) However, the latter substance was isolated in such small yields that it is not certain whether this androgen was present as a normal metabolite or whether it arose as a result of the administered testosterone (XXV)

The isolation of urinary 17-ketosteroids after the administration of testosterone (XXV) to a variety of subjects and species is summarized in Table XXV For these studies hypogonadal and castrated men a normal woman the pregnant monkey, the male chimpanzee, and the male guinea pig have been employed Four 17 ketosteroids have been identified $\Delta^{2 \text{ or } 3}$-androstenone 17 (XXVI, page 487) (an artifact of androsterone) androsterone (I, page 483), isoandrosterone (XIV, page 483) and etiocholanol 3(α) one 17 (XVIII, page 483) The conversion of testosterone to androsterone (I) has been realized in hypogonadal and castrated men a normal woman, the pregnant monkey and the male chimpanzee Etiocholanol 3(α) one-17 (XVIII) has been isolated after treatment with testosterone (XXV) in male hypogonads, a normal woman, and the male chimpanzee In one experiment in a hypogonadal man, isoandrosterone has been isolated

The administration of testosterone (XXV) to male guinea pigs resulted in the excretion of isoandrosterone (XIV) (60) Even after the administration of androsterone (I) isoandrosterone (XIV) was recovered in the urine (70)

TABLE XXIV

ISOLATION OF 17 KETOSTEROIDS AFTER ADMINISTRATION OF TESTOSTERONE PROPIONATE

Subject	Testosterone propionate administered mg *	Route of administration	17 Ketosteroids isolated in %				Ref
			Δ 2 or 3 androstenedione-17	Androsterone	Isoandrosterone	Etiocholanol 3(α) one-17	
Male hypogonad	501	Oral		8			59
Male hypogonad	300	I M		9			59
Male castrate	250	Oral		6			56a
Male hypogonad	543	I M		11		10 7	27
Male Hypogonad	1002	I M		15		4 0	56a
Rhesus monkey (pregnant)	1002	Subcu		2 2			116a
Male guinea pig	668	Subcu			2 4		60
Male hypogonad	300	I M			11 7		57
Male chimpanzee	2500	Oral	4 7	2 1		0 8	80
Normal human female	1002	I M	3 4	14 6		10 1	198

* Expressed as free testosterone

In bile fistula dogs (173) it was found that a single injection of androsterone testosterone or methyltestosterone was followed by excretion of androgenic material in the bile Oral administration of large amounts of testosterone propionate to a woman with a bile fistula did not result in significant excretion of androgens or 17 ketosteroids in the bile but the usual increased urinary excretion of these constituents was found (72)

Study of the metabolism of testosterone (XXV) and the three isomers androsterone (I) etiocholanol 3(α) one-17 (XVIII) and isoandrosterone (XIV) reveals a total of twelve possible intermediates (Figs 18 19 and 20) Only five direct intermediates have been studied in addition to testosterone and androsterone

In Fig 18 we have represented the six possible direct intermediates between testosterone (XXV) and androsterone (I) Three of the possible intermediates have been studied in hypogonadal men with the demonstration that they are converted to androsterone (I) Thus the administration of androstanediol 3(α) 17(α) (XLIII) to a 26-year old eunuchoid and to a surgically castrated male yielded androsterone (I) in both cases The administration of both Δ^4-androstenedione (XXII)

and androstanedione-3,17 (LXX) to a eunuchoid yielded androsterone (I) In a more recent study it was found that intramuscular administration of Δ^4-androstenedione-3,17 (XXII) to a fifty-year-old male showing the Laurence-Moon-Beedl syndrome gave rise in the urine to

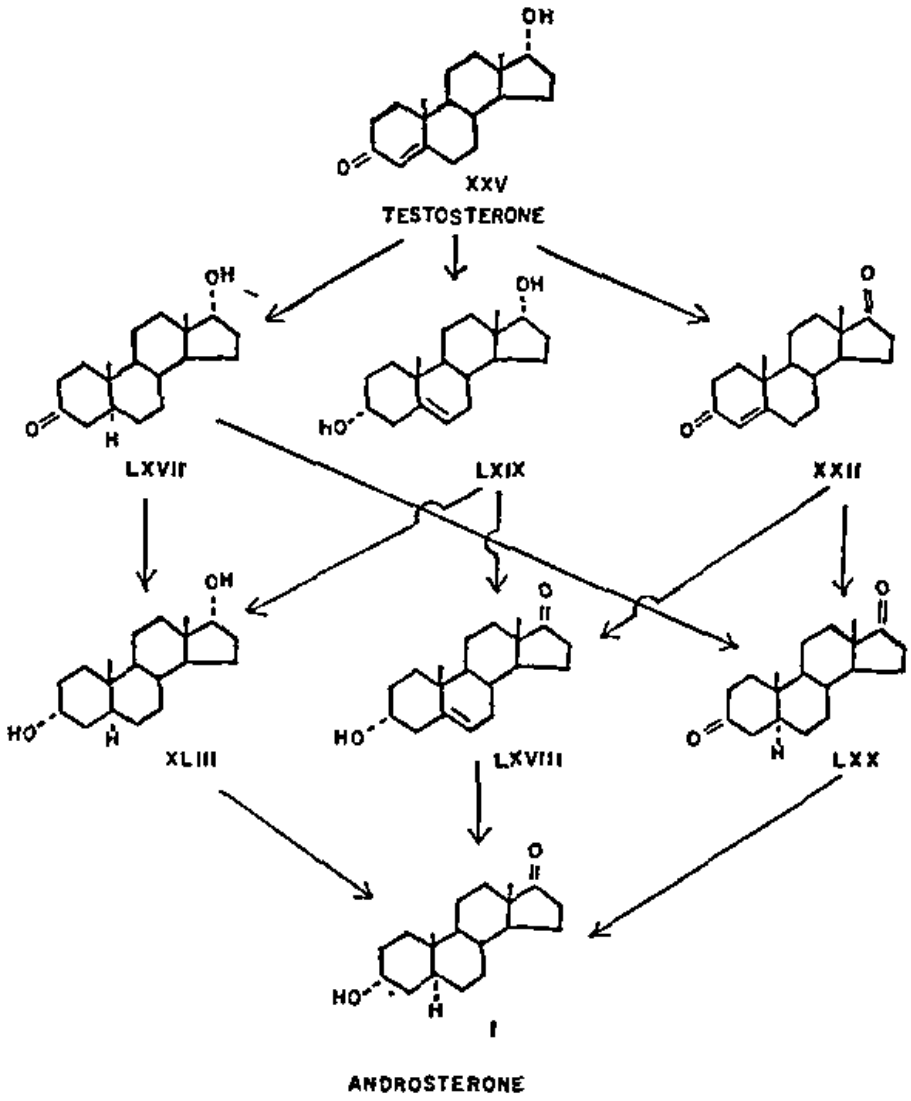


FIG 18 —Possible direct intermediates between testosterone and androsterone

the excretion of large amounts of $\Delta^{2 \text{ or } 3}$-androsterone-17, androsterone (I), and etiocholanol-3(α)-one-17 (XVIII) (73)

The six possible direct isomers between testosterone (XXV) and etiocholanol-3(α)-one-17 (XVIII) are represented in Fig 19 The administration of both testosterone (XXV) and Δ^4-androstenedione-3,17 gave rise to etiocholanol-3(α)-one-17

Fig 20 illustrates the theoretical direct conversion of testosterone (XXV) to isoandrosterone (XIV) One of the possible pathways is

through dehydroisoandrosterone (III) That this may happen seems unlikely since the administration of dehydroisoandrosterone (III) to men, guinea pigs, and rabbits has not resulted in the excretion of isoandrosterone (XIV) but instead to androsterone (I), etiocholanol-3(α)-17-one (XVIII), and Δ⁵-androstenediol-3(β),17(α) (XXVIII)

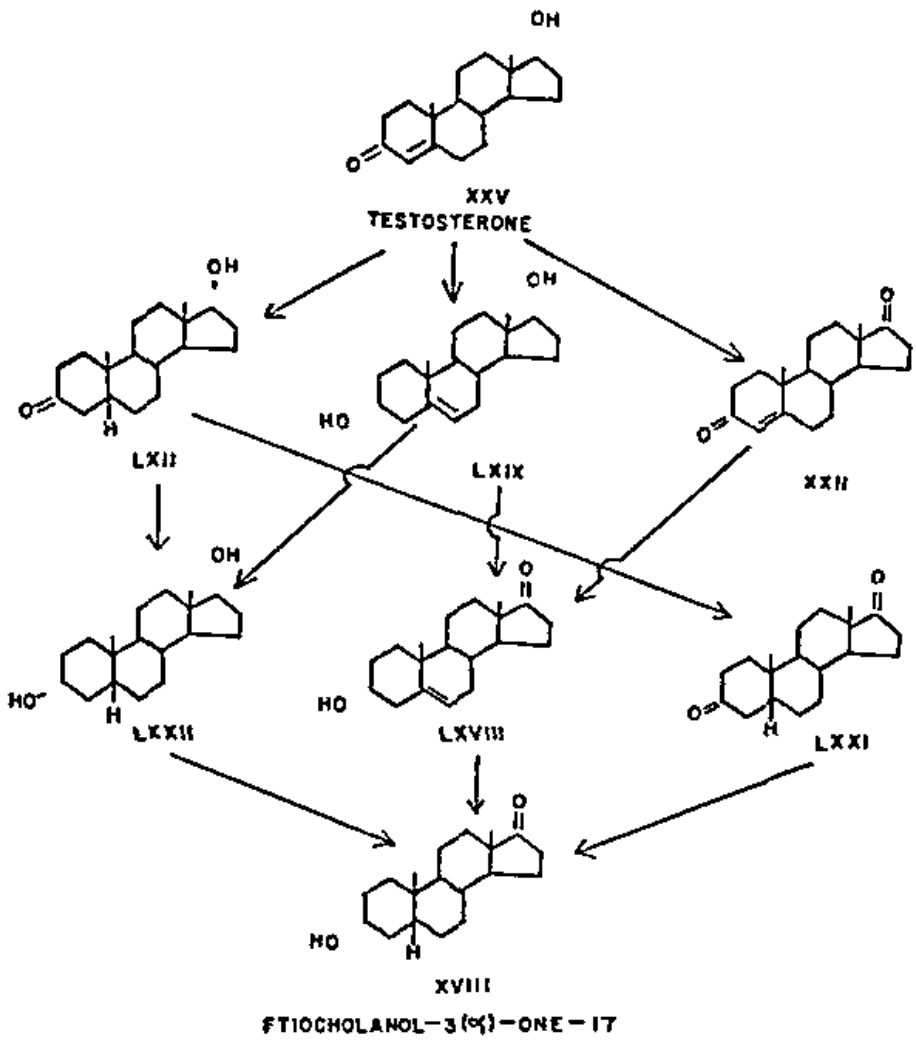


FIG 19.—Possible direct intermediates between testosterone and etiocholanol 3(α)-one-17

The twelve direct possible intermediates between testosterone (XXV) and androsterone (I), etiocholanol 3(α) one-17 (XVIII), and isoandrosterone (XIV) are dehydroisoandrosterone (III), Δ⁴ androstenedione-3,17 (XXII), Δ⁵ androstenediol 3(β),17(α) (XXVIII), androstanediol- 3(α), 17(α) (XLIII), androstanol-17(α) one-3 (LXVII), Δ⁵ androstenol 3(α) one-17 (LXVIII), Δ⁵-androstenediol-3(α),17(α) (LXIX), androstanedione 3,17 (LXX), etiocholanedione-3,17 (LXXI), etiocholanol 17 (α) one

(LXXII), etiocholanediol 3(α),17(α) (LXXIII), and androstanediol-3(β),17(α) (CX)

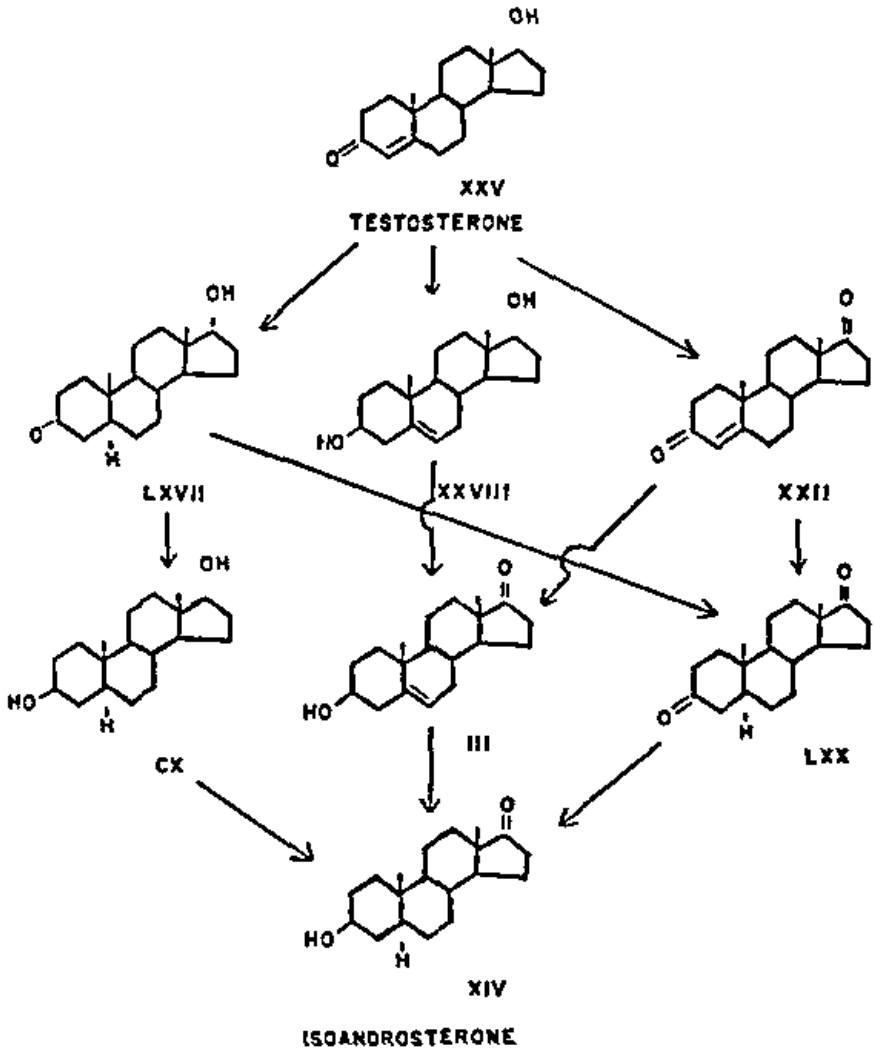


FIG 20 —Possible direct intermediates between testosterone and isoandrosterone

2 *Significance of* Δ^5*-Stenols*

The metabolism of dehydroisoandrosterone to Δ^5-androstenediol-3(β),-17(α) has been shown to be reversible Thus, in the guinea pig it has been shown that Δ^4-androstenediol-3(β),17(α) can be converted to dehydroisoandrosterone (152) Dehydroisoandrosterone can be converted to Δ^5 androstenediol 3(β),17(α) in man (114,143,154) and also to etiocholanol-3(α) one-17 and androsterone (I) (Fig 21)

An outline of the interrelation between dehydroisoandrosterone (III) and testosterone (XXV) in humans is represented in Fig 22 Δ^4-Androstenedione-3 17 (XXII) appears to play the role of the common intermediate probably being derived metabolically from both dehydroisoandrosterone (III) and testosterone (XXV) All three presumably

would occur in the body A relatively small amount of dehydroisoandrosterone (III) finds its way into the urine The bulk of the dehydroisoandrosterone (III) is converted to androsterone (I) and etiocholanol-3(α)-one-17, the latter two compounds being excreted in the urine

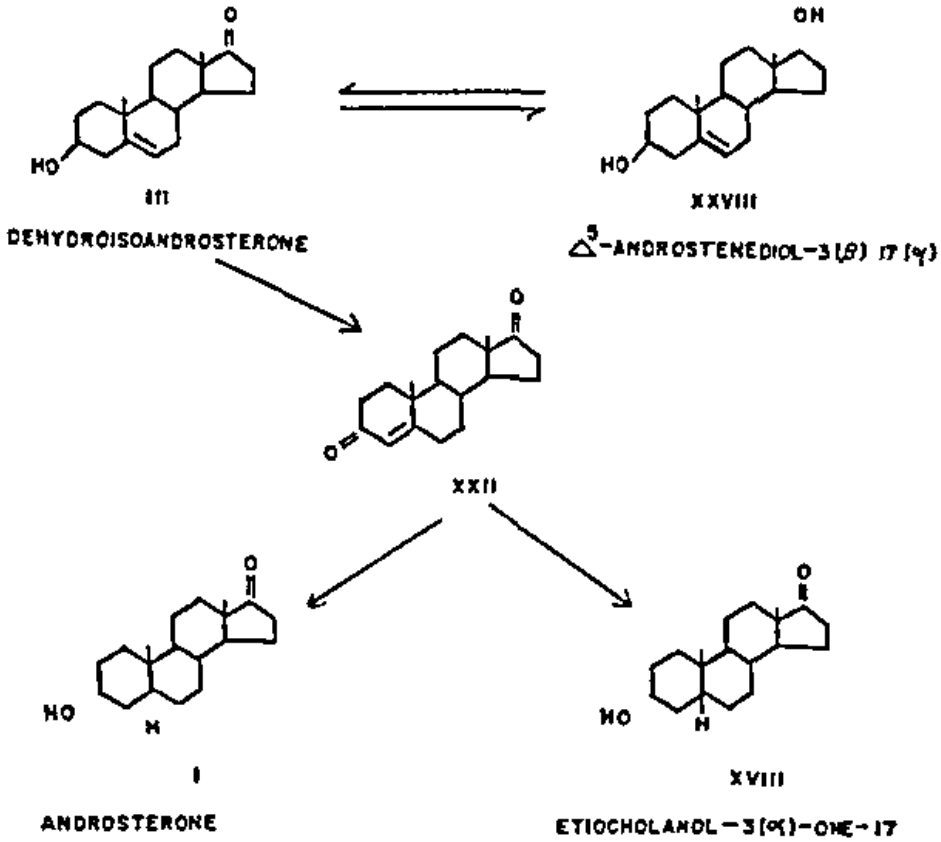


FIG 21 —Some metabolic conversions of Δ5-3(β)-stenols

Δ6 Androstenetriol-3(β) 16 17 (XLIV) has been isolated from urine, but its metabolic precursor is not known Mason reports the isolation of a minute amount of the triol after the administration of dehydroisoandrosterone (III) It has been suggested that the triol may be metabolically related to dehydroisoandrosterone in a manner similar to that previously observed for estriol and estrone (111,112)

3 *Possible Conversion of Androgens to Phenolic Estrogens*

Steinach, Kun, and Peczenik (204) originally reported the appearance of estrogenic material in the urine of normal and castrated rats after the administration of urinary extracts and androsterone In a later paper, Steinach and Kun (203) were able to increase the excretion of estrogenic material in the urine of men after the injection of testosterone propionate In one case, they reported an increase from 36 to 1200 R U /l after the injection of 1 g testosterone propionate during a six-

week period These assays were done on the total fraction Subsequent investigators (29,65,117) have shown that the increase in estrogens after testosterone therapy is in fact present in the phenolic fraction The effect can be produced in the absence of the testis Whether the effect

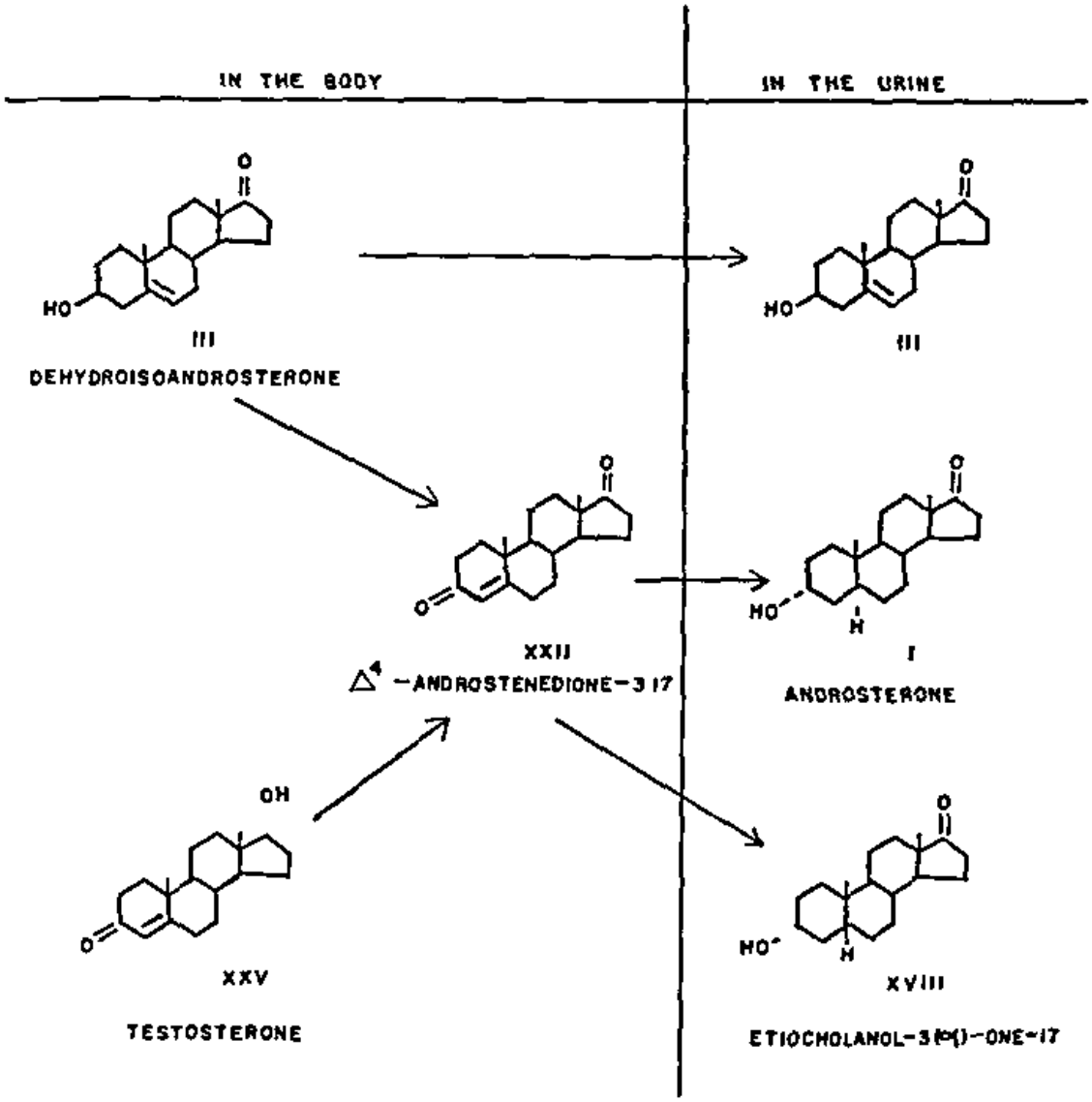


FIG 22 —Metabolic interrelations between dehydroisoandrosterone and testosterone

is by way of the adrenal cortex is of course open to question Laroche, Simmonet, and Bompard (124) have claimed that no such transformation occurs when testosterone propionate is administered to ovariectomized women

4 *Possible Conversion of Adrenal Cortical Steroids to 17-Ketosteroids*

Some 27 neutral steroids have been isolated from the adrenal cortex (167) From the available evidence, particularly from studies on urines

of patients with increased adrenal cortical activity and isolations from the urines of gonadectomized individuals, it appears that 17-ketosteroids such as androsterone, etiocholanolone, dehydroisoandrosterone, isoandrosterone, and 11-hydroxyandrosterone are derived, at least in part, from some of these adrenal cortical steroids

The question of 17-ketosteroids arising from C_{21} compounds of the adrenal cortex is still a question more of speculation than experimental verification It is well known that the adrenal cortex contains primarily two kinds of compounds one, the 3(β)-hydroxyallo, and the second the Δ^4-stenone type It has been suggested by Hirschmann and Hirschmann (112,113) that the Δ^5-stenols may be converted by dismutation to 3(β)-hydroxyallo compounds and Δ^4-stenones The Δ^4-stenone would be metabolized in a fashion similar to that of testosterone However, the question of the fate of the 3(β)-hydroxyallo compounds is still open.

If we permit ourselves to make three assumptions, the metabolism of adrenal cortical steroids to 17-ketosteroids can be considerably simplified These assumptions are as follows (*1*) that the body is capable of rupturing the 17,20-glycol or 17,20(α)-ketol with the formation of a 17-ketone, (*2*) that the body is capable of converting a 3(β) hydroxyallo compound into a Δ^5-stenone, and (*3*) that the body is capable of reducing the 11-hydroxy group to the hydrocarbon In some of the following discussions all these assumptions are employed In others the second assumption is not utilized.

a 5-Oxygen Compounds Having a 3(β)-Hydroxyallo Configuration. Fig 23 illustrates the possible metabolism of four 5-oxygen-substituted adrenal steroids to 17-ketosteroids The four compounds differ only in the state of oxidation of the carbons at C-11 and C-20, being hydroxy substitutions or ketones On removal of the side chain there is formed either 11-hydroxyisoandrosterone (XXIII) or 11-ketoisoandrosterone (LX) Assuming that the 3(β)-hydroxyallo compounds may be desaturated to Δ^5-3(β) hydroxy compounds, 11 hydroxydehydroisoandrosterone (LXI) and 11-ketodehydroisoandrosterone (LXII) would be formed Removal of the hydroxy group at C-11 will convert 11-hydroxy*dehydroisoandrosterone (LXI) to dehydroisoandrosterone (III)*, which can be partly excreted unchanged and partly metabolized to androsterone (I) and etiocholanol-3(α)-17-one (XVIII), probably by way of Δ^4-androstenedione-3,17 (XXII) The latter steroid has previously been discussed as a common intermediate in the metabolism of both dehydroisoandrosterone (III) and testosterone (XXV).

If the hydroxy group at 11 is not reduced, the 11-hydroxydehydroisoandrosterone (LXI) may be oxidized to Δ^4 androstenol-11-dione-3,17 (LXIII) with subsequent reduction to 11-hydroxyandrosterone (XL), an

androgen isolated from human urine, and etiocholanediol-3(α),11-one-17 (LXIV), a compound as yet not isolated from urine Another possibility would be a similar set of metabolic reactions as described except for the presence of an 11-ketone derivative in place of the 11 hydroxysteroid The end product under these conditions would be 11 ketoandrosterone

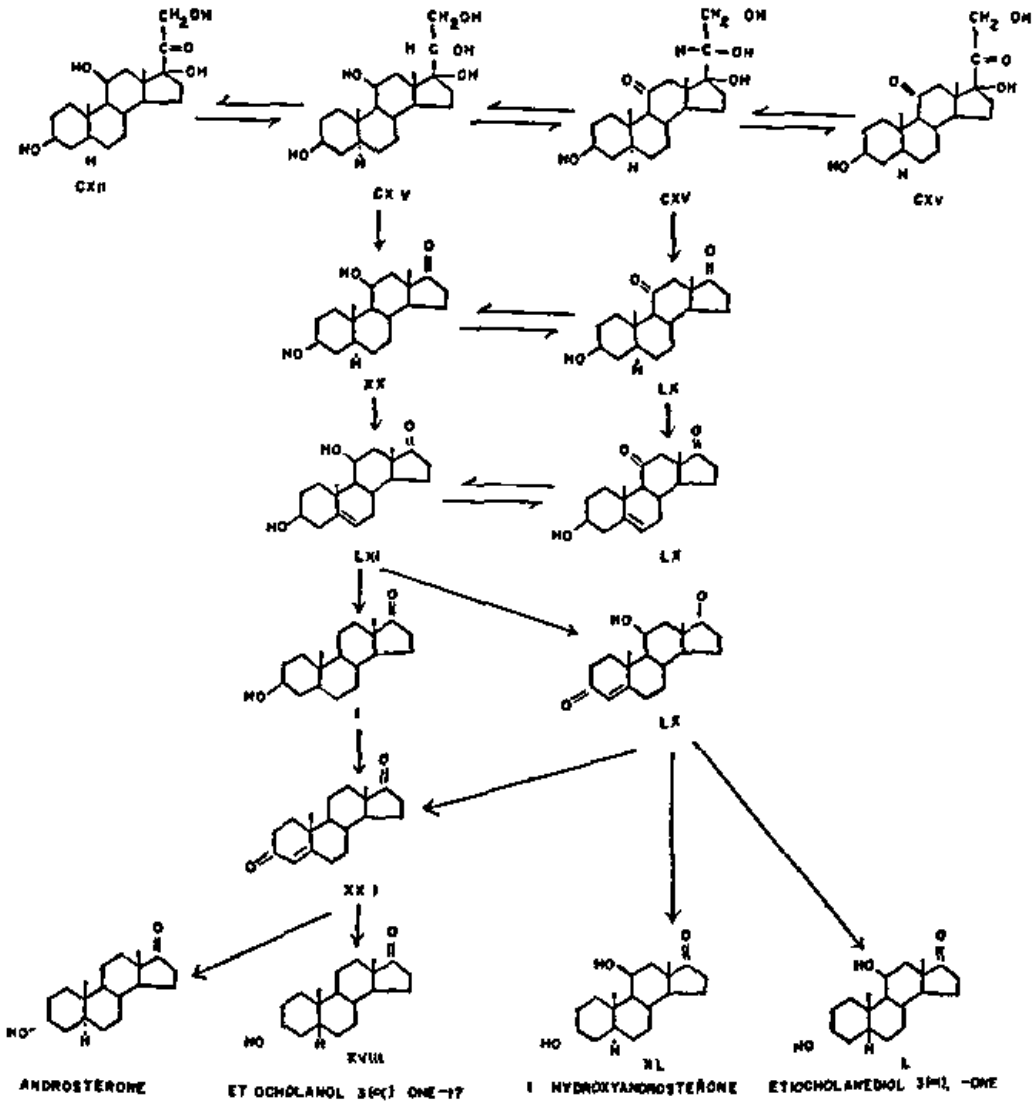


FIG 23 —Possible metabolism of adrenal cortical steroids to 17 ketosteroids
For (L) read (LXIV)

(LXV) and the etiocholanol-3(α) dione-11,17 (LXVI) The latter two steroids have not as yet been isolated from urine

If only two assumptions are made (1) that the glycol or (α) ketol configuration can be ruptured to form 17-ketosteroids and (2) that the 11 oxygen substitution can be reduced to the hydrocarbon, an alternative scheme for the metabolism of the 3(β) hydroxyallo compounds can be postulated (Fig 24) Under these conditions androsterone (I) and 11-hydroxyandrosterone (XL) would be the end metabolites

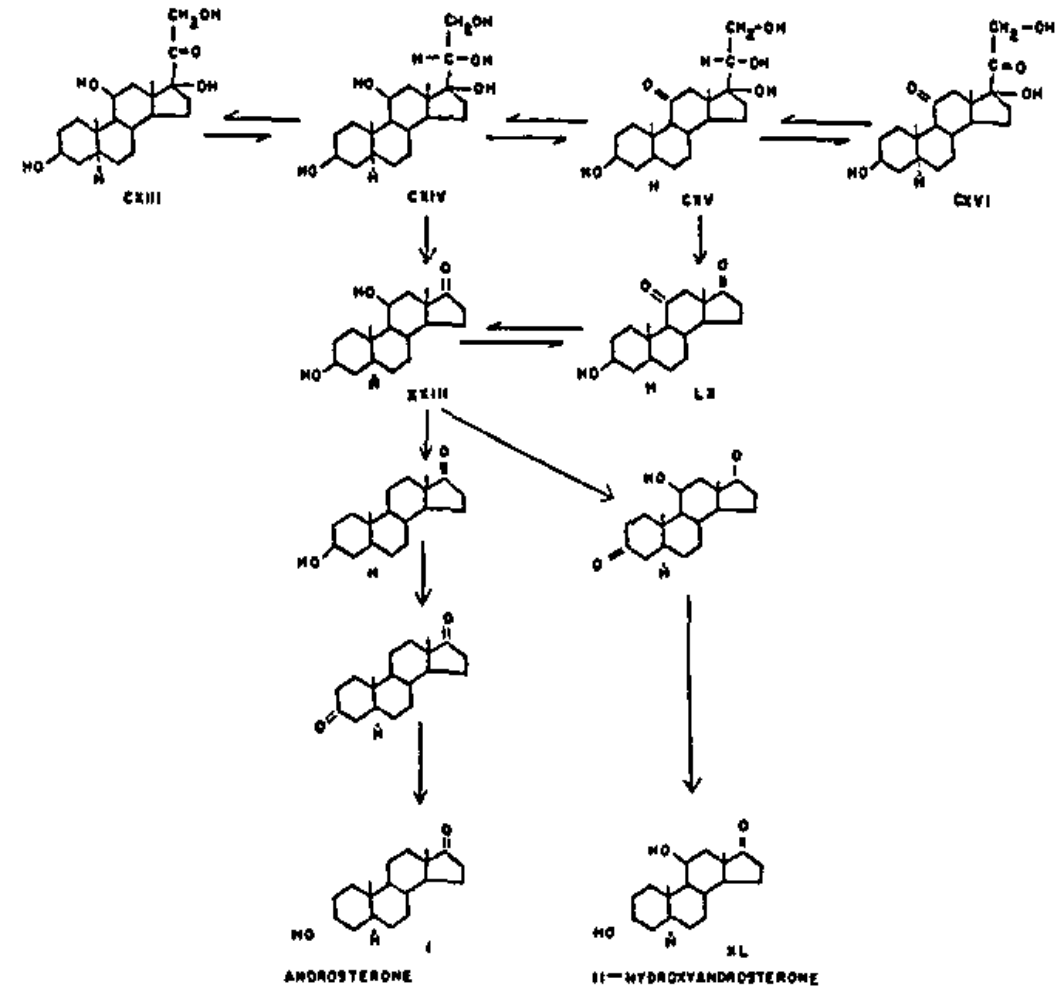


FIG 24 —Possible metabolism of adrenal cortical steroids to 17-ketosteroids

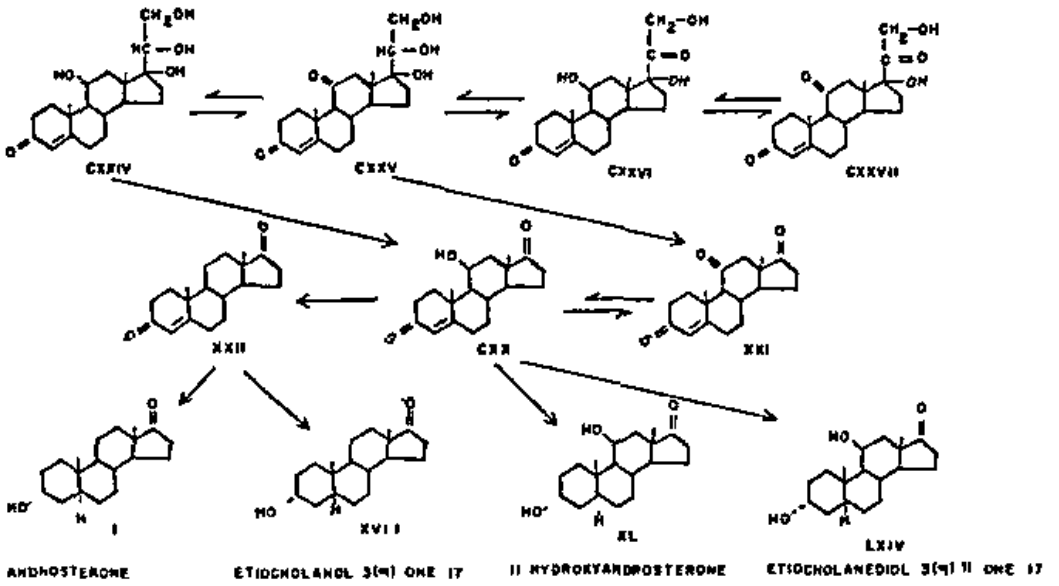


FIG 25 —Possible metabolism of adrenal cortical steroids to 17-ketosteroids

b 5 Oxygen-Δ^4-stenone Compounds Four adrenal steroids which also contain 5-oxygen substitutions, but having the Δ^4-stenone configuration, are represented in Fig 25 The reduction of the α,β unsaturated ketone would presumably be effected in a manner similar to that for testosterone (XXV) Postulating reactions at C-11 and on the side chain similar to that discussed previously, the end products should be the same as previously described in Fig 23

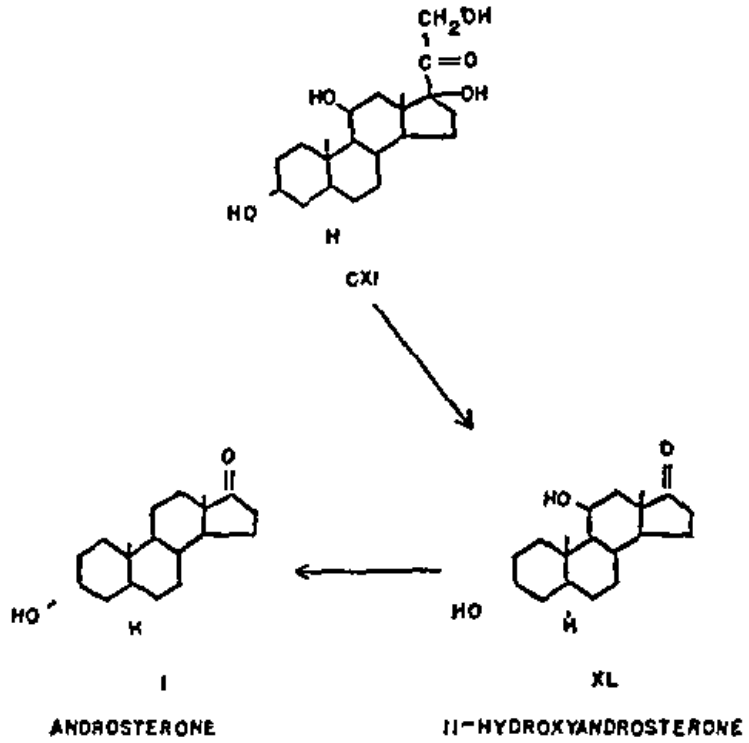


FIG 26 —Possible metabolism of an adrenal cortical steroid to 17 ketosteroids

c 5 Oxygen-3(α) hydroxyallo Compound One compound isolated from the adrenal cortex contains the saturated 3(α) hydroxyallo configuration This compound, allopregnanetetrol-3(α),11,17(β),21-one-20 (CXI) would yield on removal of the side chain, 11 hydroxyandrosterone (XL), which in turn may be reduced at C-11 to androsterone (I) (see Fig 26)

d 4-Oxygen Compounds Having 3(β) Hydroxyallo Configuration Fig 27 lists two steroids (LXXII, LXXII) which are 4-oxygen-3(β)-hydroxyallo compounds Rupture of the C-17 to C-20 bond would yield isoandrosterone (XIV) and presumably by the same reaction discussed for the 5 oxygen compounds, isoandrosterone may be metabolized to dehydroisoandrosterone (III), androsterone (I), and etiocholanol 3(α) one 17 (XVIII)

e 4-Oxygen Δ^4 stenone Compounds Fig 28 illustrates a problem similar to that of the second group of compounds discussed previously

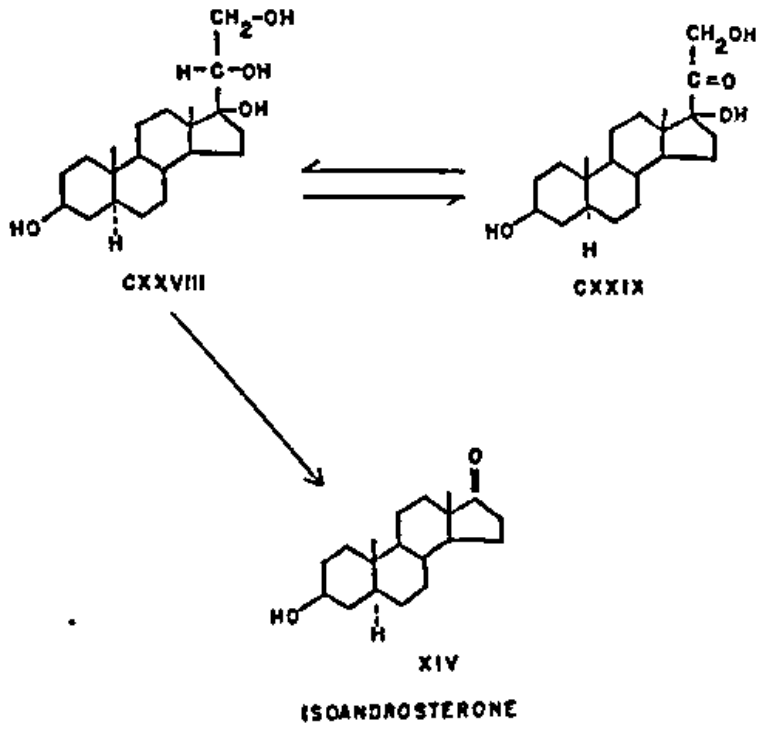


FIG 27.—Possible metabolism of adrenal cortical steroids to 17-ketosteroids

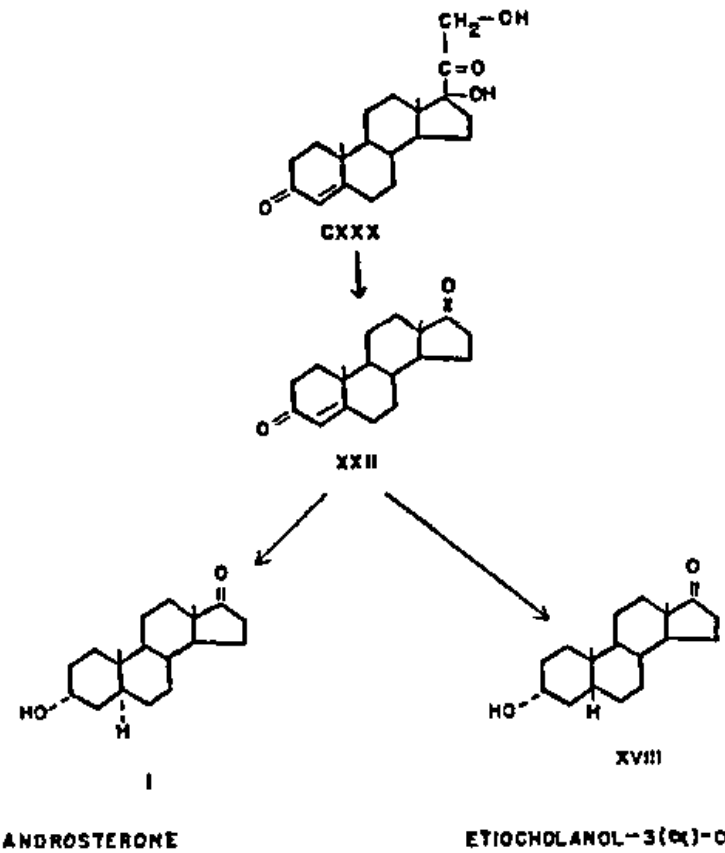


FIG 28.—Possible metabolism of an adrenal cortical steroid to 17 ketosteroids

and illustrated in Fig 25 Here we have all factors similar except that we have no oxygen substitution in ring C The simple removal of the side chain would result in the formation of Δ^4-androstenedione-3,17 (XXII), which in turn should yield androsterone (I) and etiocholano 3(α) one-17 (XVIII)

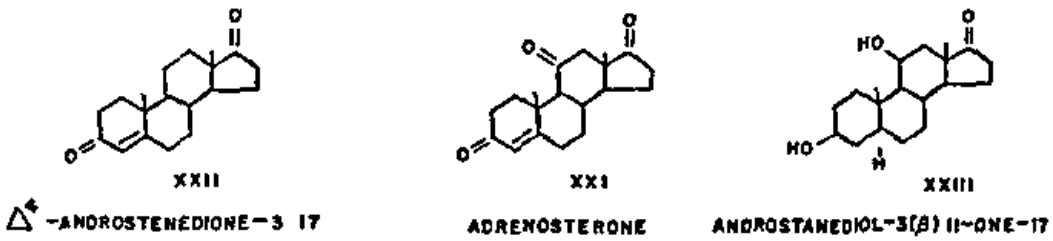


Fig 29 —17 Ketosteroids isolated from adrenal cortical tissue

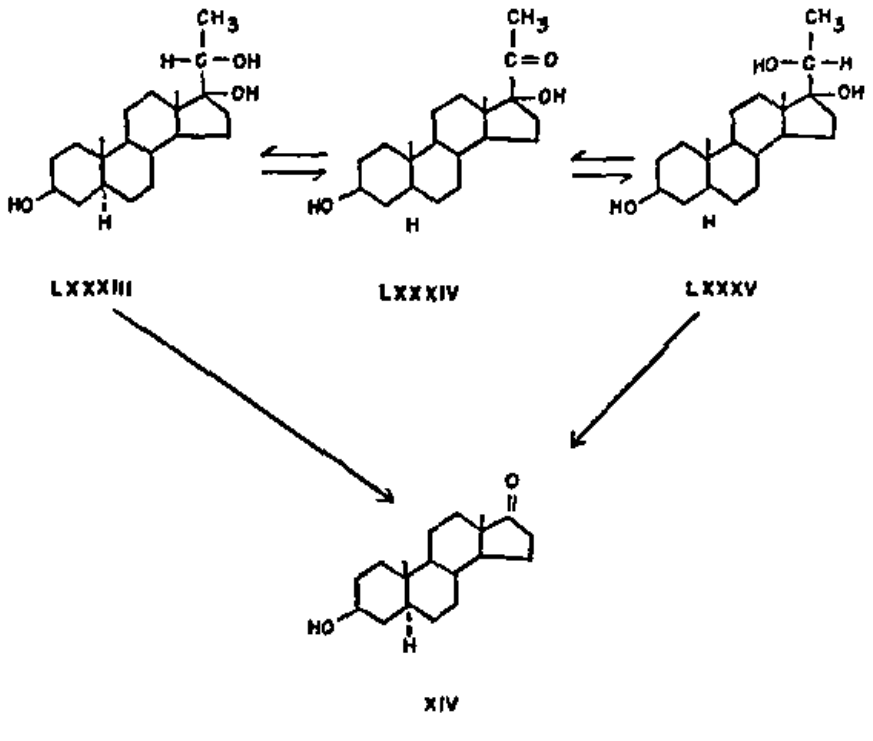


Fig 30 —Possible metabolism of adrenal cortical steroids to 17 ketosteroids

f 17-Ketosteroids in Adrenal Cortex Fig 29 illustrates three 17-ketosteroids which have been isolated from the adrenal gland Their possible place in the metabolic scheme has already been suggested It is worth remembering that Reichstein, who isolated all three of these compounds suggested the possibility that these compounds may not be present in the gland as such, but instead may be formed as a result of the isolation procedures employed

g 3 Oxygen Compounds with a 17-Hydroxy Group Figs 30 and 31 illustrate 3 oxygen-substituted adrenal steroids with 17-hydroxy groups

which should also give rise to 17-ketosteroids The compounds represented in Fig 30 have the 3(β)-hydroxyallo configuration, while the compounds represented in Fig 31 have the Δ^4-stenone configuration 17"β'-Hydroxyprogesterone (XXIV), a member of the latter group, has been shown to be an androgen

h Concerning Metabolism of Desoxycorticosterone and Adrenal Cortical Extracts to 17-Ketosteroids The administration of desoxycorticosterone to an ovariectomized chimpanzee, to an ovariectomized–adrenalectomized

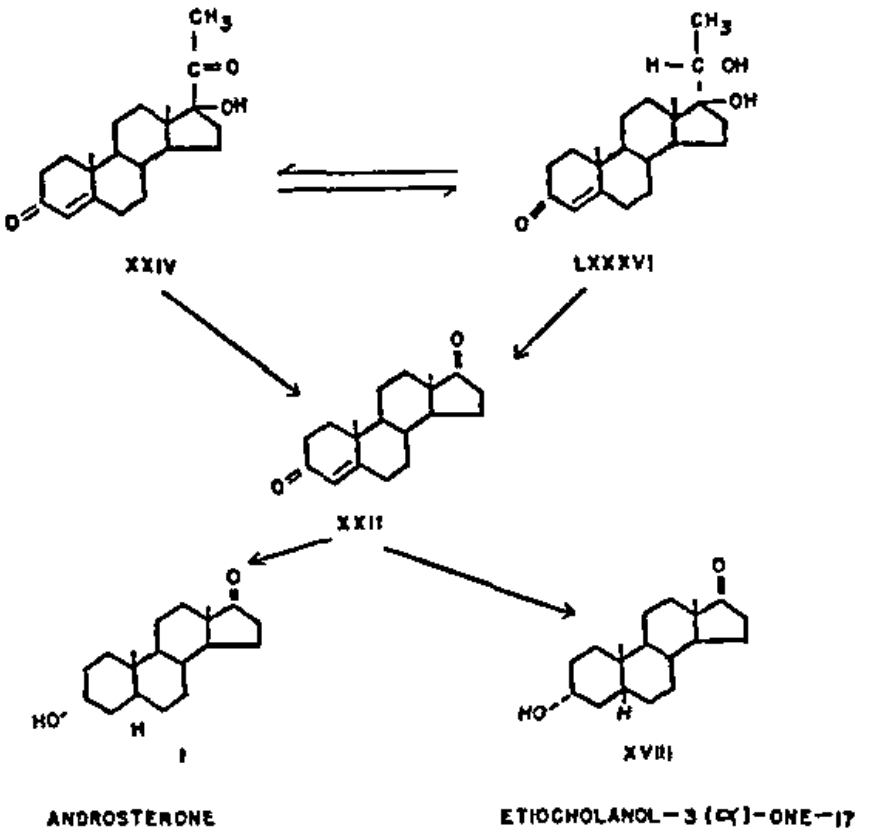


FIG 31 —Possible metabolism of adrenal cortical steroids to 17 ketosteroids

monkey, and to patients suffering from Addison's disease caused distinct increases in 17-ketosteroid excretion In the case of the Addison's disease patients, it is difficult to discern whether this increased excretion *of 17 ketosteroids was due to the general improvement of the physical* status of the patient and the secondary increased production of 17 ketosteroid precursors by the gonads, or whether a real conversion took place The former explanation seems more plausible The experiments on the ovariectomized chimpanzee and on the ovariectomized monkey indicated conversion of 3 2% to 13 8% for desoxycorticosterone to 17-ketosteroids (68)

The administration of adrenal cortical extracts to ovariectomized–adrenalectomized monkeys and to Addison's disease patients also gave rise to an increased 17-ketosteroid excretion In the monkey, calculated

minimum conversions of the adrenal cortical steroids contained in the extract to 17-ketosteroids ranged from 2.8% to 12.2%. The data for the experiments on humans are difficult to evaluate for the reason previously stated (68).

5. *Site of Inactivation of Androgens*

When pellets of testosterone or testosterone propionate are implanted in the spleen the androgenic effect on the castrated host is of a low order. The specific effect is greatly augmented if the spleen carrying the pellets is transplanted and the splenic artery and vein ligated. Thus, it appears that testosterone can be inactivated in the liver. When methyl testosterone is implanted in the spleen a greater response is elicited (5,13). It has been suggested that both methyltestosterone and testosterone are inactivated in the liver and that the two compounds show different activities when administered orally since they are absorbed by different routes from the intestinal tract (4). Methyltestosterone may be absorbed by way of the lacteals and lymphatics, thus avoiding the liver. Previously it was suggested by Miescher and Tschoop (150) that methyltestosterone suffers less destruction by intestinal enzymes than testosterone propionate.

Incubation of testosterone with rabbit liver slices in serum gave rise to Δ^4-androstenedione-3,17 and *cis*-testosterone, and incubation of Δ^4-androstenedione-3,17 under similar conditions gave rise to testosterone (38,39).

D. Enzymic Changes Due to Bacteria and Yeasts

Mamoli, Ercoli, and co-workers (78–79,129–133,199,213,214) have made extensive studies on the metabolic changes of androgens and related steroids as a result of the action of bacteria, yeasts, and molds. The work was originally undertaken to study the influence of tissues *in vitro* on the androgen, but, due to extensive contamination of tissue extracts, observed changes were due to the influence of microorganisms on the steroids.

The influence of fermenting yeast has been studied on Δ^1-androstenedione-3,17 (XCI) by Butenandt, Dannebaum, and Suranyi (20) and on androstanedione-3,17 (LXX) by Vercellone and Mamoli (213). Fig. 32 illustrates the changes observed under these experimental conditions. Both Δ^1-androstenedione-3,17 (XCI) and androstanedione-3,17 (LXX) have been reduced to androstanediol 3(β),17(α) (XCII).

Fig. 33 illustrates the changes that were effected principally by *Bacillus putrefactus*. This organism can produce reductive changes in the steroid nucleus at C-4 to the normal form, and reduce the

ketone groups at both C-3 and C-17 Thus, testosterone (XXV) may be converted to etiocholanol-17(α)-3-one (XCIII) and etiocholanediol 3(α),17(α) (XCIV) Δ^4-Androstanedione has been converted to etiocholanol-17(α)-3-one (XCIII), etiocholanediol-3(α),17(α) (XCIV), and etiocholanedione-3,17 (LXXI) Finally, it has been demonstrated that androstanedione-3,17 (LXX) can be reduced to isoandrosterone (XIV) and androstanediol-3(β),17(α) (XCII)

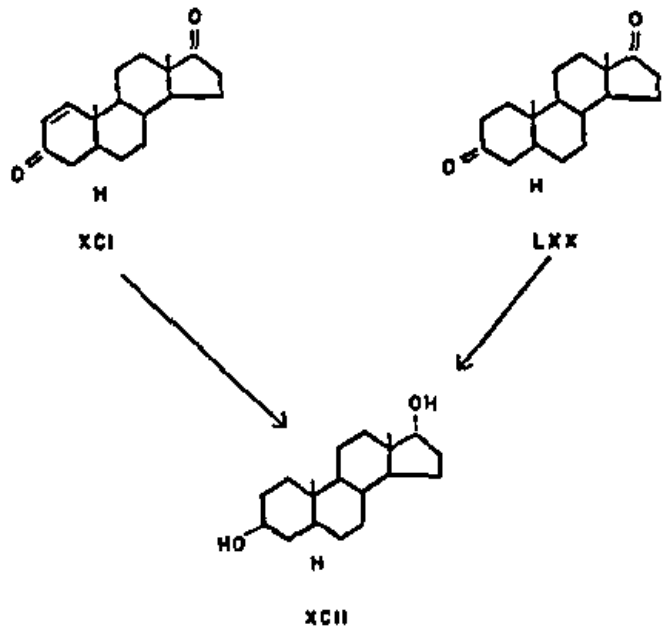


FIG 32 —Conversions under the influence of fermenting yeast

Corynebacterium mediolanum has been studied with respect to its ability to oxidize the Δ^5-stenols grouping to Δ^4-stenones In Fig 34 the conversions of dehydroisoandrosterone (III) to Δ^4-androstenedione-3,17 (XXII) and Δ^5-methylandrostenediol-3(β),17(α) (XCV) to methyltestosterone (XCVI) are illustrated That this reaction probably also takes place in the animal organism is illustrated by the conversion of dehydroisoandrosterone (III) to androsterone (I) and etiocholanol-3-Δ^5-(α)-one-17(XVIII), which probably takes place by way of Δ^4 androstenedione-3,17(XXII)

In the presence of yeast which was probably contaminated with bacteria, it has been shown that both dehydroisoandrosterone (III) and androstenediol-3(β),17(α) (XXVIII) may be converted to Δ^4-androstenedione 3,17 (XXII) These changes are illustrated in Fig 35

VII. Mechanism of Action of Androgens

Although it appears logical to assume that the mechanism of action of androgens involves enzyme or enzyme system relationships, only preliminary information on this point is available at the present time The

principal effects of androgens may be said to involve growth stimulation Thus the influences on such specific tissues as seminal vesicles, combs, and prostates are primarily growth phenomena The nitrogen-retaining properties of the androgens may also be linked to the growth process The nitrogen retention found under the influence of androgens is greater

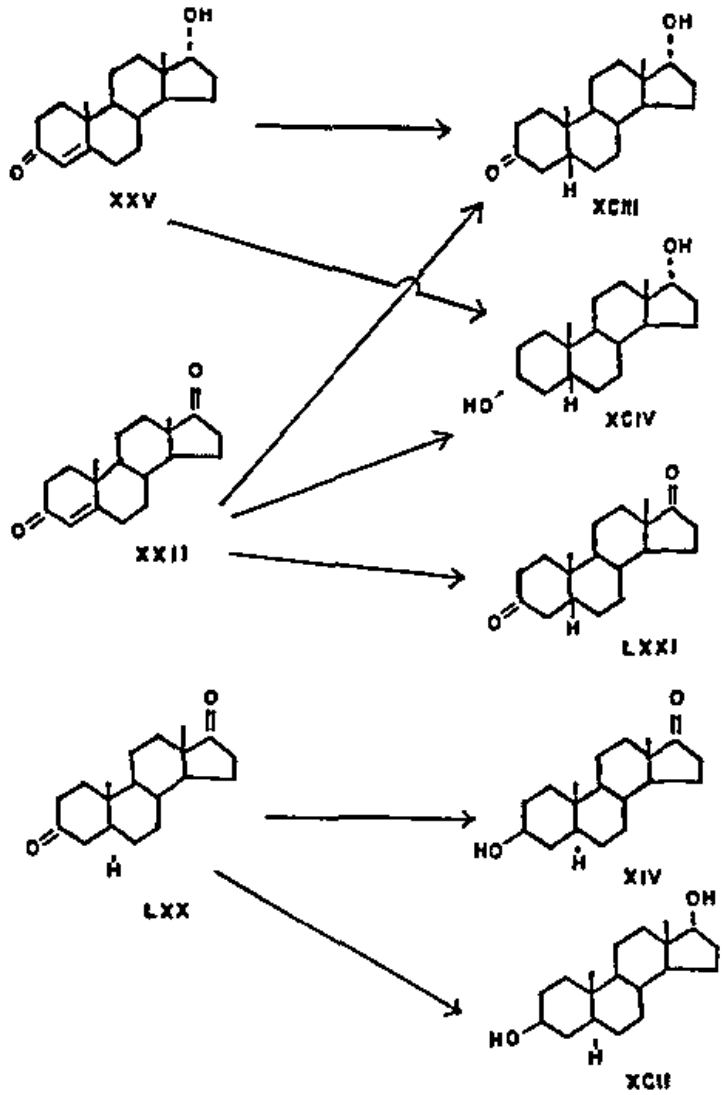
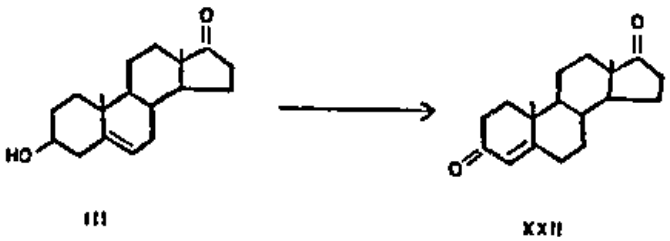


FIG 33 —Conversions under the influence of *Bacillus putrefactus* (with yeast brei)

than can be expected on the basis of sex specific tissue Rather it appears more like a general effect on the growth process of many tissues However, a second character of androgen stimulation may be related to the question of blood flow and vascularization Thus an early change in such an organ as the comb is the increased blood supply to the structure under the influence of the male sex hormone The mechanism of this increased vascularity has not been elucidated

Concerning the second phenomenon, that of growth, a number of

possible mechanisms may be mentioned First a system may be visualized whereby the androgen acts as a part of an enzyme system favoring, perhaps, growth of the organ Second, the androgens may act to remove an inhibitor.



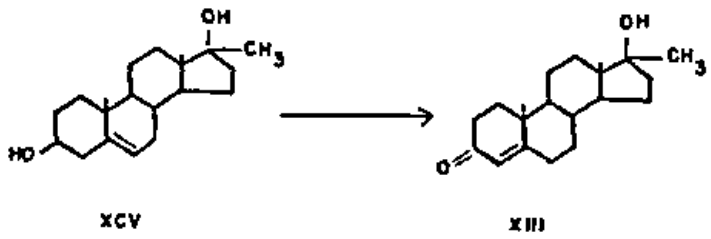


FIG 34 —Conversions due to *Corynebacterium mediolanum*

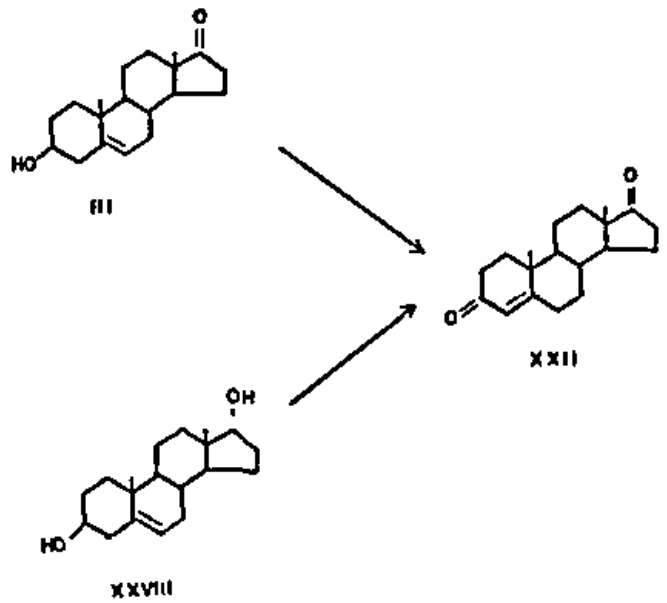


FIG 35 —Conversions due to yeast contaminated with bacteria.

Although the mechanism of action of androgens is obscure, some of the necessary conditions for this action have been described. Vitamin E deficiency in capons has been shown to prevent the full action of androgens on the comb It has been claimed that vitamin E enhances the action of small doses of androgens on the capon's comb (34)

The influence of testosterone propionate on rachitic rats has been studied (37) On a rachitic diet the secondary sex glands become atrophic, and the administration of testosterone propionate for 3 to 21 days produces characteristic responses toward the normal glands In addition to effects on secondary sex characteristics, there was observed a beneficial effect on the weights of the treated animals but no effect on the bone lesions On the rachitic diet androgens still exerted their characteristic action

The relation of the thyroid to the action of androgens on the capon's comb has been studied (34,35) There are claims that the administration of thyroxine either orally or intramuscularly intensifies and prolongs the effect of testosterone on the capon comb, while thyroidectomy reduces the comb sensitivity to androgens

VIII Inhibitory Effects of Compounds on Action of Androgens

The ability of estrogens to inhibit the biological action of androgenic substances has been established on the fowl Regnier (187) showed that estradiol benzoate caused distinct retardation of the comb in cockerels and involution of the organ in the cock The complicating factor of influence of the estrogens on the pituitary is undesirable in these experiments In later experiments the pituitary factor was removed Muhlbock (160) was able to show that 0 4 μg testosterone applied to the capon s comb caused significant growth When the same amount of testosterone was employed along with 500 μg estrone or estradiol, an inhibition of growth resulted Similar results were obtained with progesterone but not with estriol, equilenin, or Δ^5-pregnenol-3(β) one-20 In a second series of experiments, Muhlbock was able to suppress the action of parenterally administered androsterone by estrogens applied directly to the comb In the latter experiments, both estradiol benzoate and progesterone suppressed the action of androsterone

The experiments of Hoskins and Koch (118) show results similar to those found by Muhlbock Here simultaneous administration of androsterone and estrone to capons produced combs which were significantly smaller than when androsterone alone was administered Apparently specific inhibition was produced when androsterone was injected and estrone applied directly to the comb in a 60% ethanol solution

In another study Muhlbock (161) was able to show that even the involuted comb of the capon can be further suppressed by the injunction of estrogens It is quite likely that the activity of adrenal androgens is suppressed under these conditions

IX Ability of Androgens to Inhibit Action of Other Steroid Hormones

With adequate androgen concentrations it is possible to inhibit the action of other steroid hormones The effect of androgens on estrogens has been studied in detail by Robson (190) This investigator has shown that as little as 20 μg /day of testosterone can inhibit the estrus cycle of mice This action, however, cannot be considered a direct inhibition but is due most likely to the suppressing action of androgen on the pituitary resulting in decreased estrogen production of the ovary In ovariectomized mice such an effect is removed It has been shown that the vaginal cornification action of estrone may be inhibited by the simultaneous administration of such androgens as testosterone androstanediol, and androstenedione Dehydroisoandrosterone on the other hand, appeared to augment rather than inhibit the action of the estrogens Testosterone proved to be the most effective, showing the characteristic effect at a ratio of 250 to 1 Inhibition by androgens of the estrogenic effects of estradiol and triphenylethylene were also observed (191)

In the experiments mentioned one further variable must be accounted for, the adrenal cortex The possibility still exists that, in dealing with minimum amounts of estrogens to produce vaginal cornification, the effect is due to the summation of the estrogen of the adrenal plus the exogenous hormone The administration of the androgens may depress the adrenal bringing the total concentration of available estrogens below the minimal effective level An answer to this question must await similar studies on adrenalectomized ovariectomized animals

X Possible Androgenic Activity of Other Hormones and Pregnenimolone

A large number of androgenic substances which are not naturally occurring have been synthesized These steroid compounds are closely related to testosterone (200) In addition to this class of compounds, claims of androgenic activity have been made for progesterone, pregnenimolone and desoxycorticosterone

Progesterone has been claimed to be inactive by Albrieux, Bruno, Engel, and Morato (2), by Desseau (53), and by Deselm (51) Evidence for androgenic activity of progesterone has been presented by Greene, Burrell, and Thomas (94) who showed increases up to 400% in the weights of the prostates of immature castrated rats which received 2 mg of progesterone for ten days It was also possible to show significant stimulations of the prostates of adrenalectomized castrated immature rats However, since the total dosage was 20 mg of material it still is

likely that a contaminant of perhaps as little as 1% may be responsible for these androgenic effects Other reports have been published claiming androgenic activity for progesterone when administered at levels of 20 to 30 mg /day (41,119)

Pregnenmolone has been claimed to possess androgenic activity under various conditions in castrated male rats (41), and in a *Lebistes reticulatus* (186) In one experiment pregnenmolone was administered to the tadpoles of *Rana pipiens* from the early larval stage through metamorphoses It did not effect larval growth or differentiation but markedly affected sexual development All treated tadpoles became males, and genetic females could not be identified This action is identical with that found with testosterone

Whether desoxycorticosterone is androgenically active is still the subject of some debate Negative results on the capon's comb (33,134) and the castrated male rat have been reported (201) On the other hand, androgenic activity has been reported for desoxycorticosterone in castrated male rats (35,41)

REFERENCES

1 Adler, A A *Nature* **133**, 798 (1934)
2 Albrieux, A F , Bruno, W , Engel, P , and Morato, G *Klin Wochschr* **15**, 206 (1943)
2a Anderson A F , Hain, A M , and Patterson, J *J Path and Bact* **55**, 341 (1943)
3 Battelli, F *Compt rend soc phy hist nat Geneve* **39**, 73 (1922)
3a Baumann, E J , and Metzger, N *Endocrinology* **27**, 664 (1940)
4 Biskind, G R *Proc Soc Exptl Biol Med* **43**, 259 (1940)
5 Biskind, G R , and Mark, J *Bull Johns Hopkins Hosp* **65**, 212 (1939)
6 Bliss, C I *Science* **100**, 577 (1944)
7 Blyth, J S S , Dodds, E C , and Gallimore, E J *J Physiol* **73**, 136 (1931)
8 Bruger, M Rosenkrantz, J A , and Lowenstein, B F *Am J Med Sci* **208**, 212 (1944)
9 Buchheim, J *Compt rend soc biol* **109**, 1290 (1932)
10 Bulbring, E , and Burns, J A *J Physiol* **85**, 320 (1935)
11 Burrill, M W , and Greene, R R *Proc Soc Exptl Biol Med* **40**, 327 (1939)
12 Burrill, M W , and Greene, R R *Endocrinology* **26**, 645 (1940)
13 Burrill, M W , and Greene, R R *ibid* **31**, 73 (1942)
13a Burrows H Cook, J W , Roe, E M F and Warren, F L *Biochem J* **31**, 950 (1937)
14 Burrows W H, Byerly, T C , and Evans, E I *Proc Soc Exptl Biol Med* **35**, 30 (1936)
15 Busquet, H *Compt rend soc biol* **97**, 1463 (1927)
16 Busquet H *ibid* **99**, 1855 (1928)
17 Butenandt, A *Z angew Chem* **45**, 655 (1932)
18 Butenandt, A , and Dannenbaum, H *Z physiol Chem* **229**, 192 (1934)
19 Butenandt, A , Dannenbaum, H , Hanisch, G , and Kudszus, H *ibid* **237**, 57 (1935)
20 Butenandt, A , Dannenbaum, H , and Suranyi, L *Ber* **73**, 818 (1940).

21. Butenandt, A , and Hanisch, G *Z physiol Chem* **237**, 89 (1935)
22 Butenandt, A , and Kudszus, H *ibid* **237**, 75 (1935)
23 Butenandt, A and Tscherning, K *Z angew Chem* **44**, 905 (1931)
24 Butenandt, A , and Tscherning, K *Z physiol Chem* **229**, 167 (1934)
25 Butenandt, A , and Tscherning, K *ibid* **229**, 185 (1934)
26 Butler, G C , and Marrian, G F *J Biol Chem* **119**, 565 (1937)
27 Callow, N H *Biochem J* **33**, 559 (1939)
28 Callow, N H., Callow, R K , and Emmens, C W *ibid* **32**, 1312 (1938)
28a Callow, N H , Callow, R K , and Emmens, C W *J Endocrinol* **1**, 76 (1939)
29 Callow, N H , Callow, R K , and Emmens, C W *J Endocrinol* **1**, 99 (1939)
29a Callow, N H , Callow, R K , and Emmens C W *J Endocrinol* **2**, 88 (1940)
30 Callow, R. K *J Soc Chem Ind* **55**, 1030 (1936)
31 Callow, R. K *Proc Roy Soc London* **36**, 841 (1938)
31a Callow, R K , and Crooke, A C *Lancet* **246**, 464 (1944)
32 Callow, R K , and Deanesly, R *Biochem J* **29**, 1424 (1935)
33 Caridroit, F *Compt rend soc biol* **134**, 450 (1940)
34 Caridroit, F *Rev sci* **80**, 31 (1942)
35 Caridroit, F , and Aruy, L *Compt rend soc biol* **136**, 339 (1942)
36 Caridroit, F , and Regnier, V *ibid* **79**, 309 (1941)
37 Chiancone, F M *Arch ist biochim ital* **9**, 325 (1937)
38 Clark, L C , Jr , and Kochakian, C D *Endocrinology* **35**, 222 (1944)
39 Clark, L C , Jr , and Kochakian, C D Am Chem Soc Meeting Sept (1944)
40 Corner, G W *Am J Obstet Gynecol* **47**, 670 (1944)
41 Courrier, R , and Bennetz, H *Ann endocrinol* **3**, 118 (1942)
41a Crooke, A. C , and Callow, R K *Quart J Med* **32**, 233 (1939)
42 Cunningham, B , and Kuhn, H H *Proc Soc Exptl Biol Med* **48**, 314 (1941)
43 Dalmer, O , Werder, F V , Honigmann, H , and Heyns, H *Ber* **68B**, 1814 (1935)
44 Danby, M *Acta Brevia Neerl and Physiol Pharmacol Microbiol* **10**, 56 (1940)
45 Danby, M *Endocrinology* **27**, 236 (1940)
46 David, K , Dingemanse, E , Freud, J , and Laqueur, E *Z physiol Chem* **233**, 281 (1935)
47 Davidson, C S *Proc Soc Exptl Biol Med* **36**, 703 (1937)
48 Deanesly, R. *Proc Roy Soc London* **B126**, 122 (1938)
49 Deanesly, R , and Parkes, A. S *Lancet* **230**, 837 (1936)
50 Deanesly, R., and Parkes, A S *Proc Roy Soc London* **B124**, 279 (1937)
51 Desclin, L *Compt rend soc biol* **132**, 43 (1939)
52 Dessau, F *Acta Brevia Neerland Physiol Pharmacol Microbiol* **5**, 139 (1935)
53 Dessau, F *ibid* **7**, 126 (1937)
53a Dingemanse, E , Borchart, H , and Laqueur, L *Biochem J* **31**, 59 (1937)
54 Dirscherl, W *Z physiol Chem* **237**, 52 (1935)
55 Dobriner, K., Rhoads, C P , Lieberman, S , Hill, B R , and Fieser, L F *Science* **99**, 494 (1944)
56 Dorfman, R. I *J Biol Chem* **123**, 30 (1938)
56a Dorfman, R I *Proc Soc Exptl Biol Med* **45**, 739 (1940)
57 Dorfman, R. I *Proc Soc Exptl Biol Med* **46**, 351 (1941)
58 Dorfman, R. I (Unpublished) (1946)
59 Dorfman, R I , Cook, J W , and Hamilton, J B *J Biol Chem* **130**, 285 (1939)
60 Dorfman, R I , and Fish, W R *ibid* **135**, 349 (1940)

61 Dorfman, R I, and Greulich, W W *Yale J Biol Med* **10**, 79 (1937).
61a Dorfman, R I, and Gargill, S Unpublished (1946)
62 Dorfman, R I, Greulich, W W, and Solomon, C I *Endocrinology* **21**, 741 (1937)
63 Dorfman, R I, and Hamilton, J B *J Clin Investigation* **18**, 67 (1939)
64 Dorfman, R I, and Hamilton, J B *Endocrinology* **25**, 28 (1939)
65 Dorfman, R I, and Hamilton, J B *ibid* **25**, 33 (1939)
66 Dorfman, R I, and Hamilton, J B *J Biol Chem* **133**, 753 (1940).
67 Dorfman, R I, and Hamilton, J B *J Clin Endocrinol* **1**, 352 (1941)
68 Dorfman, R I, Horwitt, B N, Shipley, R A, Fish, W R, and Abbott, W E (Unpublished) (1946)
68a Dorfman, R I, Peters, J P, and Wilson, H M *Endocrinology* **27**, 1 (1940)
69 Dorfman, R I, Rubin, B, and Miller, M (Unpublished) (1946)
70 Dorfman, R I, Schiller, S, and Fish, W R *Endocrinology* **36**, 349 (1945)
71 Dorfman, R I, Schiller, S, and Sevringhaus, E L *ibid* **37**, 262 (1945)
71a Dorfman, R I, and Shipley, R A Unpublished (1946)
72 Dorfman, R I, Shipley, R A, and Wise, J E (Unpublished) (1946)
72a Dorfman, R I, and Van Wagenen, G *Proc Soc Exptl Biol Med* **39**, 35 (1938)
73 Dorfman, R I, and Van Wagenen, G *Surg Gyn Obstet* **73**, 545 (1941)
74 Dorfman, R I, Wise, J E, and McCullagh, E P (Unpublished) (1946)
75 Emmens, C W *Endocrinology* **28**, 633 (1941)
76 Engstrom, W W, and Mason, H L *ibid* **33**, 229 (1943)
77 Engstrom, W W, Mason, H L, and Kepler, E J *J Clin Endocrinol* **4**, 152 (1944)
78 Ercoli, A *Ber* **71**, 650 (1938)
79 Ercoli, A *ibid* **72**, 190 (1939)
80 Fish, W R, and Dorfman, R I *Endocrinology* **35**, 22 (1944)
81 Fish, W R, Young, W C, and Dorfman, R I *ibid* **28**, 585 (1941)
82 Frank, R T, and Klempner, E *Proc Soc Exptl Biol Med* **36**, 763 (1937)
83 Frank, R T, Klempner, E, and Hollander, F *ibid* **38**, 853 (1938)
84 Frank, R T, Klempner, E, and Kriss, B *Endocrinology* **31**, 63 (1942)
85 Fraser, R W, Forbes, A P, Albright, A, Sulkowitch, H, and Reifenstein, E C, Jr *J Clin Endocrinol* **1**, 234 (1941)
85a Friedgood, H B, and Whidden, H L *Endocrinology* **27**, 258 (1940)
86 Funk, C, and Harrow, B *Proc Soc Exptl Biol Med* **26**, 325 (1929)
87 Funk, C, Harrow, B, and Lejwa, A *ibid* **26**, 569 (1929)
88 Fussganger, R *Z med chem* **2**, 194 (1934)
89 Gallagher, T F, and Koch, F C *J Pharmacol* **40**, 327 (1930)
90 Gallagher, T F, and Koch, F C *J Biol Chem* **104**, 611 (1934)
91 Gallagher, T F, and Koch, F C *J Pharmacol* **55**, 97 (1935)
91a Gallagher, T F, Peterson, D H, Dorfman, R I, Kenyon, A T, and Koch, F C *J Clin Investigation* **16**, 695 (1937)
92 Gibson, J G, Jr, and Evans, W A, Jr *J Clin Investigation* **16**, 301, (1937)
93 Greene, R R, and Burrill, M W *Proc Soc Exptl Biol Med* **45**, 780 (1940).
94 Greene R R, Burrill, M W, and Thomson, D M *Endocrinology* **27**, 469 (1940)
95 Greene, R R, and Burrill, M W *ibid* **29**, 402 (1941)
96 Greenwood, A W, Blyth, J S S, and Callow, R K *Biochem J* **29**, 1400 (1935)

97 Greulich, W W, Dorfman, R I, Catchpole, H R, Solomon, C I, and Culotta, C S *Soc Res Child Development*, VII, No 3 (1942)
98 Ham, A M *Edinburgh Med J* **42**, 101 (1935)
98a Hamblen, E C, Cuyler, W K, and Baptist, M *J Clin Endocrinology* **1**, 777 (1941)
98b Hamblen, E C, Ross, R A, Cuyler, W K, Baptist, M, and Ashley, C *Endocrinology* **25**, 491 (1939)
99 Hamilton, J B, and Dorfman, R I *Endocrinology* **24**, 711 (1939)
99a Hamilton, J B, Dorfman, R I, and Hubert, G *J Lab Clin Med* **27**, 917 (1942)
99b Hansen, E H *Endocrinologie* **21**, 9 (1938)
100 Hays, H W, and Mathieson, D R *ibid* **37**, 266 (1945)
101 Heard, R D H, and Hoffman, M M *J Biol Chem* **138**, 651 (1941)
102 Heard, R. D H, and McKay, A F *ibid* **131**, 371 (1939)
103 Heard, R D H, and McKay, A F *ibid* (in press)
104 Heller, R. E *Proc Soc Exptl Biol Med* **27**, 751 (1930)
105 Heller, R E *Am J Anat* **50**, 73 (1932)
106 Hill, R T *Endocrinology* **21**, 633 (1937)
107 Hill, R T, and Gardner, W U *Anat Record* **64**, S, 21 (1936)
108 Hill, R. T, and Strong, M T *Endocrinology* **27**, 79 (1940)
109 Hirano, S O *J Pharm Soc Japan* **56**, 122 (1936)
110 Hirschmann, H *J Biol Chem* **130**, 421 (1939)
111 Hirschmann, H *ibid* **150**, 363 (1943)
112 Hirschmann, H, and Hirschmann, F B *ibid* **157**, 601 (1945)
113 Hirschmann, H, and Hirschmann, F B *ibid* **167**, 7 (1947)
114 Hoffman, M M Conference on Bone and Wound Healing 9th meeting
115 Hollander, F, and Klempner, E, and Frank, R T *Proc Soc Exptl Biol Med* **46**, 1 (1941)
116 Holtroff, A F, and Koch, F C *J Biol Chem* **135**, 377 (1940)
116a Horwitt, B N, Dorfman, R I, and Van Wagenen, G *Endocrinology* **34**, 352 (1944)
117 Hoskins, W H, Coffman, J R, Koch, F C, and Kenyon, A T *Endocrinology* **24**, 702 (1939)
118 Hoskins, W H, and Koch F C *ibid* **25**, 266 (1939)
119 Kehl, R, Paris, R, Benoit, J, and Gros, G *Compt rend soc biol* **136**, 575 (1942)
119a Kenyon, A T, Gallagher, T F, Peterson, D H, Dorfman, R I, and Koch, F C *J Clin Invest* **16**, 705 (1937)
120 Klempner, E, Hollander, F, Frank, R T, and Kriss, B *Endocrinology* **31**, 71 (1942)
121 Koch, F C *Physiol Revs* **17**, 153 (1937)
122 Koch, F C *Harvey Lectures* 205 (1937–1938)
122a Kochakian, C D *Endocrinology* **21**, 60 (1937)
123 Korenchevsky, V, and Dennison, M *Biochem J* **29**, 1720 (1935)
124 Laroche, G, Simmonet, H, and Bompard, F *Compt rend soc biol* **130**, 521 (1939)
125 Leathem, J H *Anat Record* **89**, 155 (1944)
126 Lieberman, S, and Dobriner, K *J Biol Chem* **161**, 269 (1945)
127 Loewe, S *Proc Soc Exptl Biol Med* **37**, 483 (1937)
128 Loewe, S, Voss, H F, Lange, F, and Wahner, A *Klin Wochschr* **7**, 1376 (1928)

128a Lukens F D W, and Palmer, H D *Endocrinology* **26**, 941 (1940)
129 Mamoli, L *Ber* **71**, 2278, 2696, 2701 (1938)
130 Mamoli, L *Gazz chim ital* **69**, 237 (1939)
131 Mamoli, L, Koch, R, and Teschen, H *Z physiol Chem* **261**, 287 (1939)
132 Mamoli, L, and Schramm, G *Ber* **71**, 2082, 2698 (1938)
133 Mamoli, L, and Vercellone, A *ibid* **71**, 154, 1686 (1938)
134 Manoro, J M *Arch clin inst endocrinol Montevideo* **1**, 343 (1937–1940)
135 Marker, R E *J Am Chem Soc* **57**, 1755 (1935)
136 Marker, R E *ibid* **61**, 944 (1939)
137 Marker, R E, Rohrman, E, Lawson, E J, and Wittle, E L *ibid* **60**, 1901 (1938)
138 Marker, R E, Whitmore, F C, Kamm, O, Oakwood, T S, and Blattman, J M *ibid* **58**, 338 (1936)
139 Marrian, G F *Nature* **154**, 19 (1944)
140 Marx, L *Arch Internal Med* **124**, 584 (1931)
141 Mason, H L *J Biol Chem* **158**, 719 (1945)
142 Mason, H L *ibid* **162**, 745 (1946)
143 Mason, H L, and Kepler, E J *ibid* **160**, 255 (1945)
144 Mason, H L, and Kepler, E J *ibid* **161**, 235 (1945)
145 Mathieson, D R, and Hays, H W *Endocrinology* **37**, 275 (1945)
145a McCullagh, E P *J Am Med Assoc* **112**, 1037 (1939)
145b McCullagh, E P Unpublished (1946)
146 McCullagh, D R, and Cuyler, W K *J Pharmacol* **66**, 379 (1939)
147 McCullagh, E P, McCullagh, D R, and Hicken, N F. *Endocrinology* **17**, 49 (1933)
148 McGee, L C Biological Activity of Testicular Extracts Ph D Dissertation, University of Chicago (1927)
149 Miescher, K, and Gasche, P *Schweiz med Wochschr* **72**, 279 (1942)
150 Miescher, K, and Tschopp, E *ibid* **68**, 1258 (1938)
151 Miescher, K, Wettstein, A, and Tschopp, E *Biochem. J* **30**, 1970 (1936)
152 Miller, A M, and Dorfman, R I *Endocrinology* **37**, 217 (1945)
153 Miller, A. M, Dorfman, R I, and Miller, M (Unpublished) (1946)
154 Miller, A M, Dorfman, R I, and Sevringhaus, E L *Endocrinology* **38**, 19 (1946)
155 Miller, S, and Mason, H L *J Clin Endocrinology* **5**, 220 (1945)
156 Moore, C R, and Gallagher, T F *Am J Anat* **45**, 39 (1930)
157 Moore, C R, Lamar, J K, and Beck, N *J Am Med Assoc* **111**, 11 (1938)
158 Moore, C R and McGee L C *Am J Physiol* **87**, 436 (1928)
159 Moore, C R and Samuels L T *ibid* **96**, 278 (1931)
160 Muhlbock, O *Acta Brevia Neerland Physiol Pharmacol Microbiol* **8**, 50 (1938)
161 Muhlbock, O *ibid* **8**, 142 (1938)
162 Munson P L, Gallagher, T F, and Koch, F C *J Biol Chem* **152**, 67 (1944)
162a Nathanson I T and Aub, J C *J Clin Endocrinology* **3**, 324 (1943)
163 Nathanson I T, Towne, L E and Aub J C *Endocrinology* **28**, 851 (1941)
164 Nathanson I T and Wilson, H *ibid* **33**, 189 (1943)
165 Nelson W O *Anat Record Suppl* **81**, 87 (1941)
166 Newerla G L *New Engl J Med* **228**, 40 (1943)
167 Noble G K and Wurm, M *Endocrinology* **26**, 837 (1940)
168 Oesting R B *Proc Soc Exptl Biol Med* **36**, 524 (1937)
169 Oesting, R B, and Webster, B *Endocrinology* **22**, 312 (1938)

170 Oppenauer, R V *Nature* **135**, 1039 (1935)
171 Parkes, A S *Quart J Pharm Pharmacol* **9**, 669 (1936)
172 Parkes, A S *Nature* **139**, 965 (1937)
173 Paschkis, K E, Cantarow, A, Rakoff, A E, Hansen L P, and Walkling, A A *Proc Soc Exptl Biol Med* **55**, 127 (1944)
173a Patterson, J, McPhee, I M, and Greenwood, A W *Brit Med J* **1**, 35 (1942)
174 Pearlman, W H, and Pincus, G *Vitamins and Hormones* **1**, 313 (1943)
175 Peterson, D H, Gallagher, T F and Koch, F C *J Biol Chem* **119**, 185 (1937)
176 Peterson, D H, Hoskins, W H, Coffman J R, and Koch F C *ibid* **123**, 93 (1938)
177 Pfiffner, J J, and North, H B *ibid* **132**, 459 (1940)
178 Pfiffner, J J, and North, H B *ibid* **139**, 855 (1941)
179 Pincus, G *Endocrinology* **32**, 176 (1943)
180 Pincus, G Recent Progress in Hormone Research Vol I, Academic Press New York, 1946
181 Pincus, G, and Pearlman, W H *Endocrinology* **29**, 413 (1941)
182 Prelog V, and Ruzicka L *Helv Chim Acta* **27**, 61 (1944)
183 Prelog, V, Ruzicka, L, and Steinmann, F *ibid* **27**, 674 (1944)
184 Prelog, V, Ruzicka, L, and Wieland, P *ibid* **27**, 66 (1944)
185 Price, D *Am J Anat* **60**, 79 (1936)
186 Regnier, M T *Compt rend* **213**, 537 (1941)
187 Regnier, V *Compt rend soc biol* **127**, 519 (1938)
188 Reichstein, T *Helv Chim Acta* **19**, 29 (1936)
189 Reichstein, T *ibid* **19**, 223 (1936)
190 Robson, J M *J Physiol* **92**, 371 (1938)
191 Robson, J M *Quart J Exptl Physiol* **28**, 71 (1938)
192 Ruzicka, L *Bull chim France* **5**, t2 1497 (1935)
193 Ruzicka L, Goldberg M W, Meyer, J Brungger H and Eichenberger E *Helv Chim Acta* **17**, 1395 (1934)
194 Ruzicka, L, and Prelog, V *ibid* **26**, 975 (1943)
195 Ruzicka, L, Prelog, V, and Wieland, P *ibid* **26**, 2050 (1943)
196 Ruzicka, L, and Wettstein, A *ibid* **18**, 986 (1935)
197 Salter, W T, Cahen, R L, and Sappington, T S *J Clin Endocrinol* **6**, 52 (1946)
198 Schiller, S, Dorfman, R I, and Miller, M *Endocrinology* **36**, 355 (1945)
199 Schramm, G, and Mamoli, L *Ber* **71**, 1322 (1938)
199a Scott, W W, and Vermeulen, C *J Clin Endocrinology* **2**, 450 (1942)
200 Selye, H Encyclopedia of Endocrinology Section I, Vol IV, 1943, p 14
201 Selye, H, and Albert, S *J Pharmacol* **76**, 137 (1942)
201a Simpson, H L, de Fremery, P, and Macbeth, A *Endocrinology* **20**, 363 (1936)
202 Starkey, W F, Grauer, R C, and Saier, E *Proc Soc Exptl Biol Med* **44**, 649 (1940)
203 Steinach, E, and Kun, H *Lancet* **2**, 845 (1937)
204 Steinach, E, Kun, H, and Pecznik, O *Wien Klin Wochshr* **49**, 899 and 903 (1936)
205 Tagmann, E, Prelog, V, and Ruzicka L *Helv Chim Acta* **29**, 440 (1946)
206 Talbot, N B, Berman, R A, and MacLachlan E A *J Biol Chem* **143**, 211 (1942)

207 Talbot, N B, Butler, A M, and MacLachlan, E A *ibid* **132**, 595 (1940)
207a Talbot, N B, Butler A M and Berman, R A *J Clin Investigation* **21**, 559 (1942)
207b Talbot, N B, Butler, A M, and MacLachlan, E A *New Eng J Med* **223**, 369 (1940)
208 Talbot, N B, and Ettingon, I V *ibid* **154**, 605 (1944)
209 Talbot, N B, Wolfe J K, MacLachlan, E A, and Berman, R A *ibid* **139** 521 (1941)
210 Vatna, S *Biol Bull* **58**, 322 (1930)
211 Venning E H *Endocrinology* **39**, 203 (1946)
212 Venning E H, Hoffman, M M, and Browne, J S L *J Biol Chem* **146**, 369 (1942)
213 Vercellone A and Mamoli L *Z physiol Chem* **248**, 277 (1937)
214 Vercellone, A, and Mamoli L *Ber* **71**, 152 (1938)
215 von Euw J, and Reichstein, T *Helv Chim Acta* **24**, 879 (1941)
216 Walker, C E *Proc Roy Soc Med* **1**, 153 (1908)
216a Wallis, E S and Fernholz, E *J Am Chem Soc* **57**, 1379 1504 (1935)
217 Warren F L *Cancer Research* **5**, 49 (1945)
217a Werner, S C *J Clin Investigation* **20**, 21 (1941)
218 Wolfe J K, Fieser L F, and Friedgood H B *J Am Chem Soc* **63**, 582 (1941)
219 Wolfe J K Hershberg E B, and Fieser, L F *J Biol Chem* **136**, 653 (1940)
220 Womach E G and Koch, F C *Proc Intern Congr Sex Research 2nd Congr* 329 (1930)
221 Wooley, G W and Little, C C *Cancer Research* **5**, 203 (1945)
222 Wooley, G W, and Little C C *ibid* **5**, 211 (1945)
223 Wu, H and Chou C Y *China J Physiol* **11**, 413 (1937)
224 Zimmerman W *Z physiol chem* **233**, 257 (1935)
225 Zimmerman, W *ibid* **245**, 47 (1936)

Compound Numbers

Number	Compound
I	Androsterone
II	3-Chloro Δ^5 androstenone-17
III	Dehydroisoandrosterone
IV	Dehydroisoandrosterone acetate
V	3-Chloro androstanone 17
VI	Androsterone acetate
VII	Cholesterol
VIII	Dihydrocholesterol
IX	Cholestanone
X	Epidihydrocholesterol acetate
XI	Epidihydrocholesterol
XII	Estrone
XIII	17 Methyl Δ^4 androstenol 17 one 3
XIV	Isoandrosterone
XV	Coprosterol
XVI	Etiocholanol-3(β)-one-17
XVII	Epicoprosterol
XVIII	Etiocholanol 3(α)-one 17
XIX	Δ^5 Androstenediol 3(β),17(α) 3 monoacetate

Number	Compound
XX	Δ^5-Androstenediol-3(β),17(α)-17 monobenzoate
XXI	Adrenosterone
XXII	Δ^4-Androstenedione-3,17
XXIII	Androstanediol-3(β),11-one-17
XXIV	17-Hydroxyprogesterone
XXV	Testosterone
XXVI	Δ^3 or Δ^4-Androstenone-17
XXVII	$\Delta^{3,5}$-Androstadienone-17
XXVIII	Δ^5-Androstenediol-3(β),17(α)
XL	11-Hydroxyandrosterone
XLI	Androstanetrione-3,11,17
XLII	Δ^9 or ^{11}-Androstenol-3(α)-one-17
XLIII	Androstanediol-3(α),17(α)
XLIV	Δ^5-Androstenetriol-3(β),16,17
XLV	Androstanetriol-3(β),16,17
XLVI	3-Ketoetioallobilianic acid
XLVII	16-Benzilidinandrostanol-3(β)-one-17
XLVIII	β-3-Hydroxyetioallobiliamic acid
XLIX	Δ^5-Androstenetriol-3(β),16,17-3-monoacetate
L	β-3-Hydroxy-Δ^5-etiobilienic acid
LI	Allopregnanol-3(β)-one-20-al-21
LII	Pregnanol-3(β)-one-20-al 21
LIII	Δ^5 Pregnenol-3(β)-one-20
LIV	Allopregnanol-3(β)-one-20
LV	Δ^{16}-Androstenol-3(α)
LVI	Δ^{16}-Androstenol-3(β)
LVII	Androstanol-17 (β)-one-3 hexahydrobenzoate
LVIII	Δ^{16}-Androstenone-3
LIX	Estranediol-A
LX	11-Ketoisoandrosterone
LXI	11-Hydroxydehydroisoandrosterone
LXII	11-Ketodehydroisoandrosterone
LXIII	Δ^4-Androstenol-11-dione-3,17
LXIV	Etiocholanediol 3(a), 11-one-17
LXV	11 Ketoandrosterone
LXVI	Etiocholanol-3(α)-dione-11,17
LXVII	Androstanol 17(α)-one 3
LXVIII	Δ^5-Androstenol 3(α)-one-17
LXIX	Δ^5 Androstenediol 3(α),17(α)
LXX	Androstanedione-3,17
LXXI	Etiocholanedione-3,17
LXXII	Etiocholanol-17(α)-one-3
LXXIII	Etiocholanediol-3(α),17(α)
LXXIV	Allopregnanediol-3(β),(21) dione-11,20
LXXV	Allopregnanetriol-3(β),11(β),20(α)
LXVI	Δ^5-Pregnenetriol-3(β),11(β),20(α)
LXXVII	Pregnanediol-3(α),20(α)
LXXVIII	Δ^4-Pregnenediol-20,21-dione-3,11
LXXIX	Δ^4-Pregnenediol-11(β),21-dione 3,20
LXXX	Δ^4-Pregnenol-21-trione-3,11,20

Number	Compound
LXXVI	Pregnanetriol 3(α) 11(β) 21 one 20
LXXVII	Pregnanetriol 3(α) 11(β) 20
LXXVIII	Allopregnanetriol 3(β) 17(β) 20(α)
LXXIX	Allopregnanediol 3(β) 17(β) one-20
LXXX	Allopregnanetriol 3(β) 17(β) 20(β)
LXXXVI	Δ^5 Pregnenediol 17(β) 20(α)
LXXXVII	Desoxycorticosterone
LXXXVIII	Allopregnanol 3(β)-one 20
LXXXIX	Allopregnanediol 3(β) 20(α)
XC	Progesterone
XCI	Δ^4-Androstenedione 3 17
XCII	Androstanediol 3(β) 17(α)
XCIII	Etiocholanol 17(α) one 3
XCIV	Etiocholanediol-3(α) 17(α)
XCV	Δ^6 Methylandrostenediol 3(β) 17(α)
XCVI	Methyltestosterone
XCVII	Androstanediol 3(β) 17(α)
XCIX	Etiocholanediol 3(β) 17(α)
C	Δ^5 Pregnenediol-3(β) 17 one 20
CI	Δ^5 Pregnenetriol 3(β) 17 20
CII	Allopregnanediol 3(α) 20(α)
CIII	Androsterone sulfate
CIV	Dehydroisoandrosterone benzoate
CV	Dehydroisoandrosterone sulfate
CVI	Pregnaned ol 3(α) 17 one 20
CVII	3-Chloro-Δ^5 androstenol 17(α)
CVIII	Pregnanetriol 3(α) 17 20
CIX	Δ^5 Pregnenediol 3(β) 17-one 20
CX	Androstanediol 3(β) 17(α)
CXI	Allopregnanetetrol 3(α) 11 17(β) 21-one-20
CXII	Δ^5 Pregnenediol 3(β) 20(α)
CXIII	Allopregnanetetrol 3(β) 11(β) 17(β) 21 one 20
CXIV	Allopregnanepentol-3(β) 11(β) 17(β) 20 21
CXV	Allopregnanetetrol 3(β) 17(β) 20 21-one 11
CXVI	Allopregnanetriol 3(β) 17(β) 21 dione 11 20
CXVII	Androstanol 3(β) dione-11 17
CXVIII	Δ^5 Androstenediol 3(β) 11-one-17
CXIX	Δ^5 Androstenol 3(β)-dione 11 17
CXX	Δ^4 Androstenol 11(β) dione-3 17
CXXI	Etiocholanediol 3(α) 11(β) one-17
CXXII	11 Ketoandrosterone
CXXIII	11 Ketoetiocholanol 3(α)
CXXIV	Δ^4-Pregnenetetrol 11(β) 17(β) 20 21 one-3
CXXV	Δ^4 Pregnenetriol 17(β) 20 21 d one-3 11
CXXVI	Δ^4-Pregnenetriol 11(β) 17(β) 21-dione-3 20
CXXVII	Δ^4-Pregnenediol 17(β) 21 trione-3 11 20
CXXVIII	Allopregnanetetrol 3(β) 17(β) 20 21
CXXIX	Allopregnanetriol 3(β) 17(β) 21-one-3
CXXX	Allopregnanetriol 3(β) 11(β) 21-one-20

Chapter XIII

Chemistry and Metabolism of the Adrenal Cortical Hormones[1]

By R D H HEARD

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[1] The author is much indebted to Drs J S L Browne, D A Prins and D L Thomson for considered criticism of various aspects of this review and Miss J Cohen for valuable help in the preparation of the manuscript

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I. Historical Introduction and Synopsis

The first extracts of cortical tissue which would maintain life in the adrenalectomized animal were prepared in 1930 by Hartman and Brownell (81) and by Swingle and Pfiffner (247) The active agent was designated "cortin" by Hartman and co-workers, a name which became ambiguous in meaning when subsequent investigations led to the isolation of at least six pure chemical compounds, two of which (the "desoxy" group) are highly active with respect to life maintenance and the retention of sodium and chloride ions but relatively impotent as regards carbohydrate metabolism, and four of which (the 11-oxygen group) show marked activity in the latter connection but are relatively ineffective in the survival test Further, after the separation from cortical extract of all material crystallizable by the application of the existing methods, there remains an "amorphous fraction" which retains of the order of 14–30% of the activity (based on life maintenance) of the original whole extract The term "cortin" is used today in several senses, to denote (*a*) the active substances contained in whole adrenal extract, (*b*) any life maintenance factor of unestablished chemical nature (as in the amorphous fraction), and (*c*) substances, again of unrecognized constitution, which possess any kind of cortical activity (*i e*, urinary "cortin," the active excretory products in urine, concentrates of which promote life maintenance, sodium and chloride ion retention, and gluconeogenesis)

Intensive investigation aimed at the isolation and chemical characterization of the cortical hormones was undertaken from 1934 by four investigators with their respective collaborators, E C Kendall, J J Pfiffner, and O Wintersteiner, in the United States, and T Reichstein, in Switzerland Several crystalline compounds were quickly obtained by each group, and designated, in the order of isolation, by the letters of the alphabet, but physiological activity was not clearly associated with a crystalline product until 1936, when Mason, Myers, and Kendall (167)

established the effectiveness of their Compound E (VIII, page 557) in the work performance test of Ingle (108) The same compound had also been isolated by Wintersteiner and Pfiffner—Compound F (268)—and by Reichstein—Compound Fa (205)—who found it inactive at the dose levels employed for maintenance of life in the adrenalectomized dog and recovery of fatigued muscle (Everse-de Fremery test, 54) At the same time Reichstein (205) separated in crude state his Compound H, which in 1937 was obtained in pure form (206) and shown to be highly active (65, Everse-de Fremery test), the name corticosterone was assigned There followed the elucidation of the structure of the compounds and the isolation from cortical extract, chiefly by Reichstein and collaborators, of a series of 26 crystalline steroids six of which possess marked cortical activity The first of these to be prepared artificially was 11-desoxycorticosterone (XXII, page 558) which Steiger and Reichstein (243) obtained in 1937 in fair yield from cholesterol or stigmasterol as original starting material The partial synthesis of the adrenal hormones bearing an oxygen atom in the 11 position presented serious chemical difficulties which were not overcome until 1943 when Lardon and Reichstein (127) first realized 11-dehydrocorticosterone (XVI, page 558) from desoxycholic acid in extremely minute yield

II Hormone Concentrates

In principle, the preparation of cortical extracts for experimental and clinical use involves the extraction of fresh whole adrenal glands with a neutral water miscible organic solvent, such as acetone or alcohol, followed by the separation of the hormone mixture from much inert material and from adrenaline by various solvent partition procedures The final concentrate is then adjusted to contain an arbitrarily chosen number of biological units per unit volume, or the potency is stated in terms of the equivalent weight of whole adrenal tissue

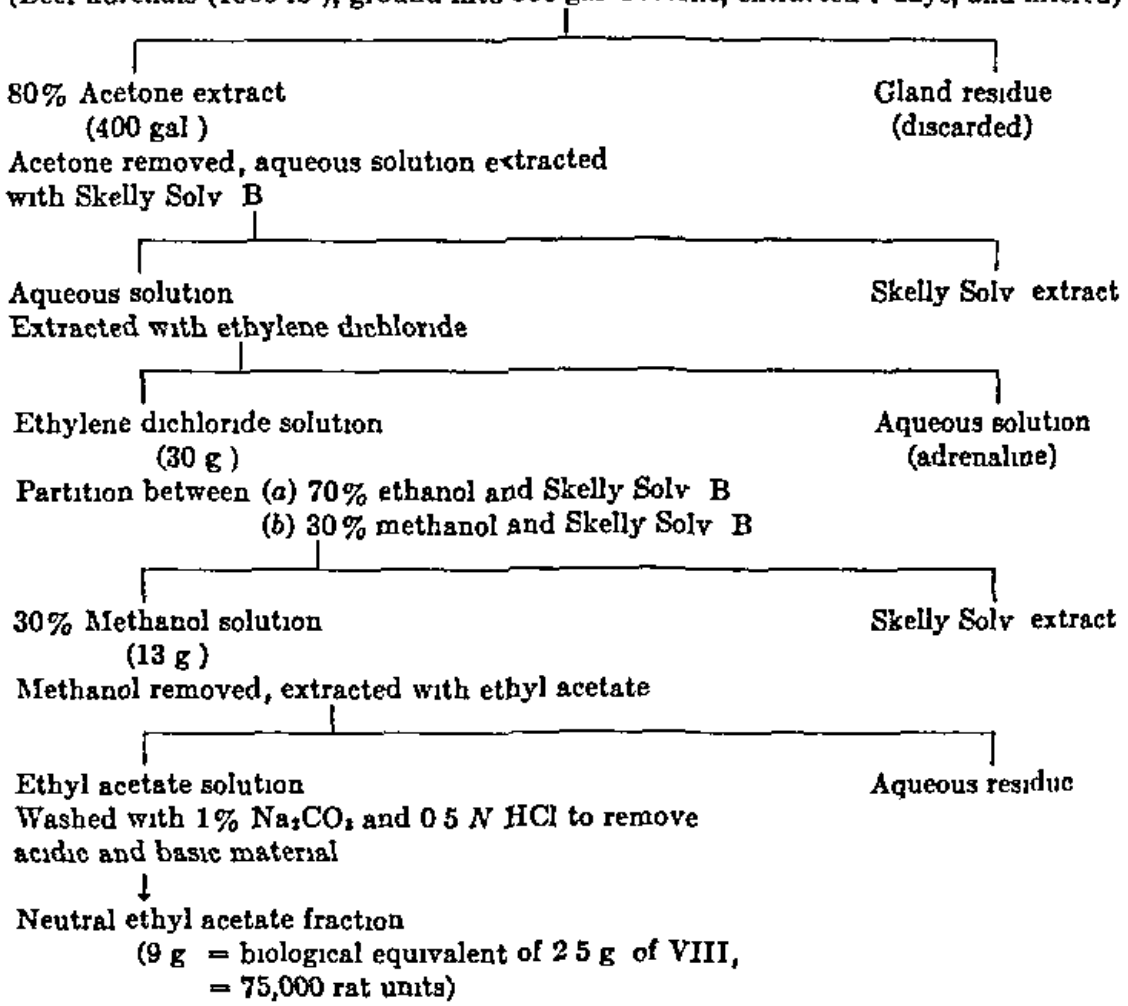
While many preparative methods are described, that of Kuizenga *et al* (120,122) suffices to illustrate (Table I) a typical process, which yields, from 1000 lb of beef adrenals, 9 g of final product containing the biological equivalent of 2 5 g of Kendall s Compound E (VIII), when compared by the work performance test of Ingle (111), or 8 rat units per mg when assayed by the survival growth test of Cartland and Kuizenga (28)

Species variation in total hormone content of the gland is considerable Based on either the work test of Ingle (111) or the time survival test (28), extracts from hog adrenals show approximately double the potency of those from beef or sheep glands (122)

(See Table IV, page 560)

TABLE I

PREPARATION OF ADRENAL CORTICAL EXTRACT



III. The Steroids Isolated in Crystalline State from Adrenal Tissue

A LIST OF COMPOUNDS

Set out in Table II and formulated[1] in Table III are the steroids isolated in crystalline state from adrenal extracts The arrangement in Table II (according to carbon and oxygen content) and the recorded physical constants are taken from Reichstein and Shoppee (212) For the complete bibliography and a full description of the individual compounds, reference should be made to this and other review articles (120,241)

The adrenal steroids lend themselves to several methods of classification, each of which possesses certain merits, both from the chemical and

[1] The configurations at C-11 and C-17 are corrected and expressed in accordance with the findings recorded later (pages 564 and 574) Some formulations therefore deviate in part from those assigned in certain of the original communications

TABLE II

STEROIDS ISOLATED FROM THE ADRENAL GLAND

Series	No	Structure and common name	Designation			Physical properties						
						Free compound			Acetylated compound			
			Reichstein *et al*	Kendall *et al*	Winterstener, Pfiffner, *et al*	M p	$[\alpha]_D$	$[\alpha]_{5461}$	Acetate	M p	$[\alpha]_D$	$[\alpha]_{5461}$
						°C (cor)	°	°		°C (cor)	°	°
$C_{21}O_5$	I	Allopregnane-3(β),11-(β),17"β",20,21-pentol	A	D	A	221–222	+16[a]	+29[a]	3,20,21	219–220	+74[a]	
	II	Allopregnane-3(β),-11(β),17"β",21-tetrol-20-one	V			220–225	+50 7[b]	+68[b]	3,21	225–227	+62 6[b]	+77 3[b]
	III	Allopregnane-3(α),-11(β),17"β",21-tetrol-20-one	C	C	D	273–276	+73[a] +59[b]	+90[a] +76[b]	3,21	204–205	+73 8[b]	+90 5[b]
	IV	Allopregnane-3(β),-17"β",21-triol-11,-20-dione	D	G	B(?)	238–242	+61 8[b] +66[a]	+78 7[b] +83[a]	3,21	223–224	+72 3[b]	+84 7[b]
	V	Δ[4] Pregnene-11(β),-17"β",20,21-tetrol-3-one	E			125 (hydrate)	+87[a]		20,21	229–230	+162 7[c]	
	VI	Δ[4] Pregnene-17"β", 20,21-triol 3,11-dione	U			208			20,21	252–253	+178 5[c]	

TABLE II (*Continued*)

Series	No	Structure and common name	Designation			Physical properties						
						Free compound			Acetylated compound			
			Reichstein *et al*	Kendall *et al*	Wintersteiner, Pfiffner, *et al*	M p	$[\alpha]_D$	$[\alpha]_{6461}$	Acetate	M p	$[\alpha]_D$	$[\alpha]_{6461}$
	VII	Δ^4 Pregnene-11(β),-17 'β' 21-triol-3,20 dione (17 Hydroxy-corticosterone)	M	F		207–210 217–220	+167 2[a]	+178[a(?)]	21	223–225		
	VIII	Δ^4 Pregnene-17 'β'',-21-diol 3,11 20-trione (17 Hydroxy-11 dehydrocortico-sterone)	Fa	E	F	215	+209[a]	+270[d] +248[a]	21	239–241		
$C_{21}O_4$	IX	Allopregnane 3(β),-17 'β'',20β,21-tetrol	K			198–200	−1[a]		3,20,21	178–179	+53 2[c]	
(a) 11-Desoxy group	X	Allopregnane 3(β),-17''β'',21 triol 20-one	P			230–239	+48[a]		3,21	208–209	+46 1[a] +28 2[c] +44 5[b]	+53 8[a] +47 4[b]
	XI	Δ^4-pregnene-17''β'',-21 diol-3 20-dione	S			213			21	239–241	+116[c]	
(b) Corticoster-one group	XII	Allopregnane 3(β),-11(β),21 triol 20-one	R			202–204			3,21	173–174	+83 7[b] +92 4[c]	+102 7[b] +114 3[c]
	XIII	Allopregnane 3(β),21-diol-11,20 dione	N	H		189–191	+93 8[a]		3,21	144–145	+77 5[c] +85 6[b]	+99 5[c] +105 6[b]
	XIV	Δ^4-Pregnene-20,21-diol-3,11 dione	T			*ca* 210 (crude)			20,21	212–213		

TABLE II (*Continued*)

Series	No	Structure and common name	Designation			Physical properties						
						Free compound			Acetylated compound			
			Reichstein *et al*	Kendall *et al.*	Wintersteiner, Pfiffner, *et al*	M p	$[\alpha]_D$	$[\alpha]_{5461}$	Acetate	M p	$[\alpha]_D$	$[\alpha]_{5461}$
	XV	Δ^4-Pregnene-11(β),21 diol 3,20 dione (Corticosterone)	H	B		180–182	+223[a]	+258[a]	21	145–146	+200[b]	+245[b]
	XVI	Δ^4-Pregnen-21-ol-3,-11,20-trione (Dehydrocorticosterone)		A		178–180		+209[a] +347[d]	21	179–181	+233 7[b]	+285 1[b]
	XVII	α,β Unsaturated ketone of unknown constitution ($C_{21}H_{28-30}O_4$)				261–264 (uncor)	+133[a]		*mono*	208–210		
$C_{21}O_2$.	XVIII	Allopregnane 3(β),-17"β",20β triol	J			216–217	−7 9[a]		3,20	159–160	+24 6[a]	
	XIX	Allopregnane 3(β),-17"β",20α triol	O			222–223	−12 6[f]		3,20	250	−30 1[a]	
	XX	Allopregnane-3(β) - 17"β"-diol-20-one	L		G	264	+38[a] +30 6[a]					
	XXI	Δ^4-Pregnen-17"β"-ol-3 20 dione (17 'β"-Hydroxyprogesterone)				222–223	+102[a] +105 6[a]					

TABLE II (*Continued*)

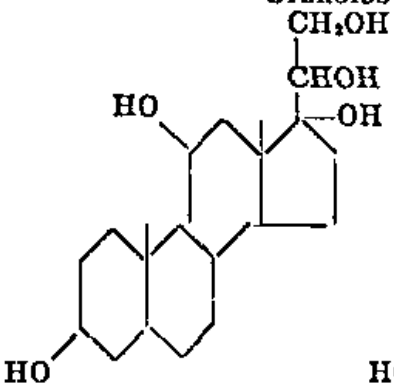
Series	No	Structure and common name	Designation			Physical properties						
						Free compound			Acetylated compound			
			Reichstein *et al*	Kendall *et al*	Wintersteiner, Pfiffner *et al*	M p	$[\alpha]_D$	$[\alpha]_{5461}$	Acetate	M p	$[\alpha]_D$	$[\alpha]_{5461}$
	XXII	Δ^4-Pregnen-21-ol 3,20-dione (Desoxycorticosterone)	Q			141–142	+178[a]		21	157–159	+177[a] +173 6[b] +164 3[c]	+211 9[b] +199 8[c]
$C_{21}O_3$	XXIII	Allopregnan 3(β)-ol-20-one				194	+70[a] +90 8[a]		3	144 5	+79 8[a]	
	XXIV	Δ^4-Pregnene 3,20-dione (Progesterone)				120, 129	+174 6[c] +193 5[c] +200[d]	+214 7[c]				
$C_{19}O_3$	XXV	Androstane-3(β),-11(β) diol 17 one				234–235	+84 5[a] +81 3[b]	+105[b]	3	228–229	+70 5[b]	+87 1[b]
	XXVI	Δ^4-Androstene 3 11,-17 trione (Adrenosterone)	G			223–224	+262[a]	+364[a]				
$C_{19}O_2$	XXVII	Δ^4-Androstene 3,17-dione				173–174	+190[a] +199[b]					
$C_{18}O_2$	XXVIII	$\Delta^{1,3,5,10}$ Estratrien 3-ol-17 one (Estrone)				255	+159[a] +170[b]	+188[a]	3	126		

[a] Ethanol [b] Dioxane [c] Acetone
[d] Benzene [e] Chloroform [f] Methanol

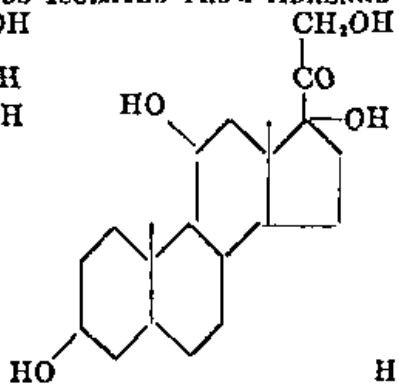
physiological point of view (*a*) With the exception of estrone (XXVIII), all contain 19 or 21 carbon atoms which admits of a C_{19} series (Compounds XXV to XXVII) and a C_{21} series (Compounds I to XXIV). Further, the C_{19} and C_{21} compounds may be subgrouped in accordance with the number of oxygen atoms inherent in the molecule (as listed in Table II) (*b*) In all instances, C-11 is either unsubstituted (the 11-desoxy series, Compounds IX–XI, XVIII–XXIV, and XXVII–XXVIII), or bears a ketonic or alcoholic function (the 11-oxygenated series, Compounds I–VIII,

TABLE III

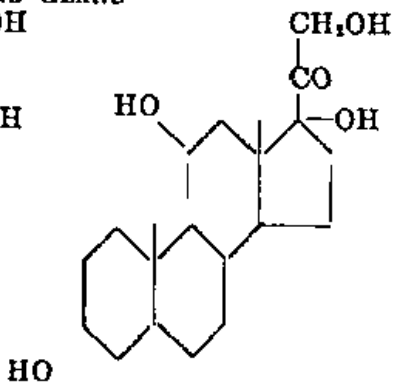
STEROIDS ISOLATED FROM ADRENAL GLAND



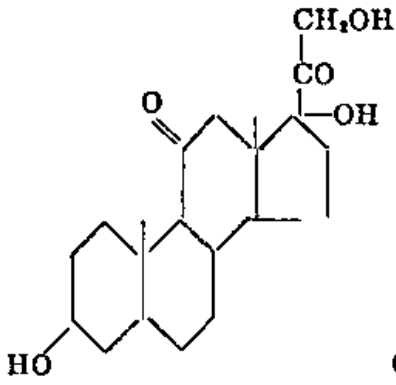
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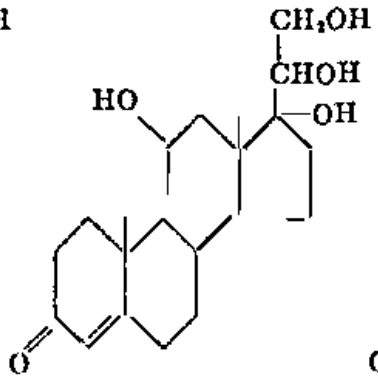
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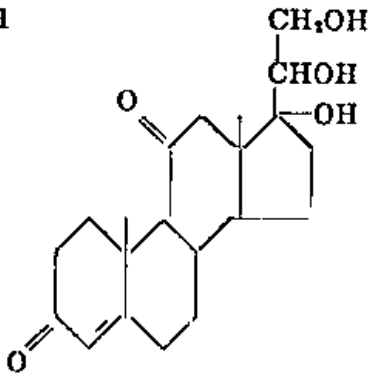
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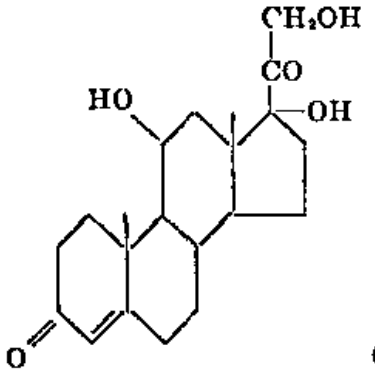
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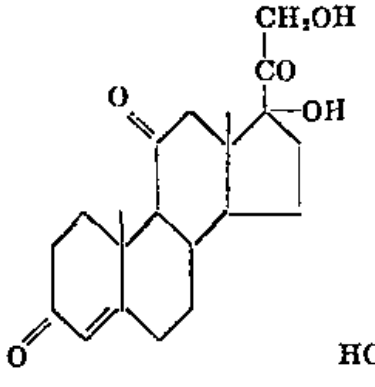
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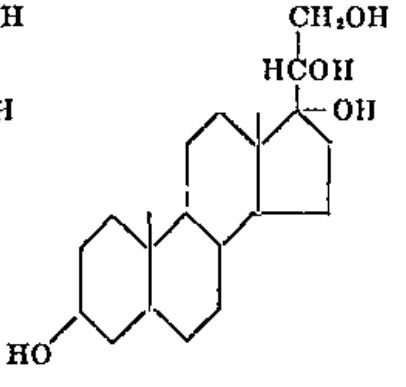
VI



VII

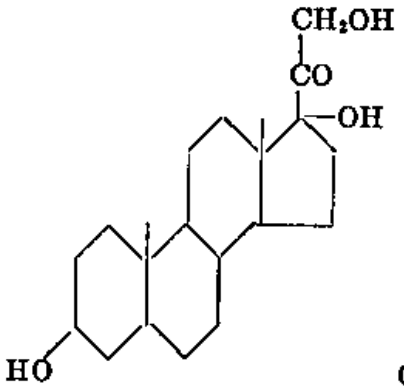


VIII

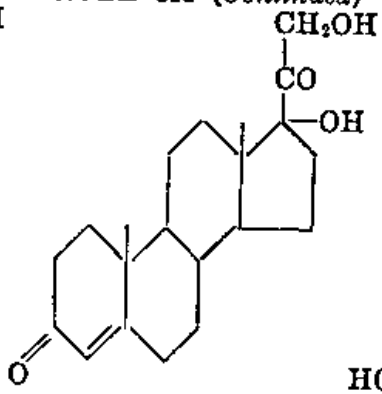


IX

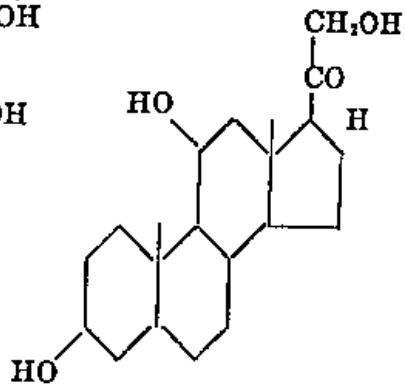
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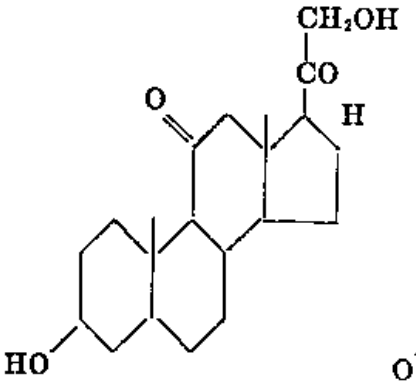
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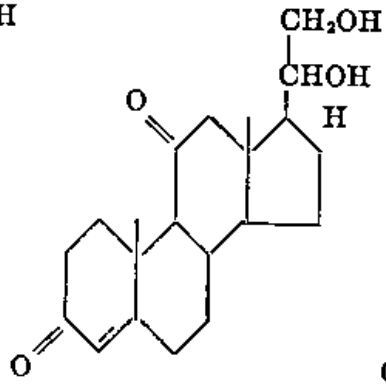
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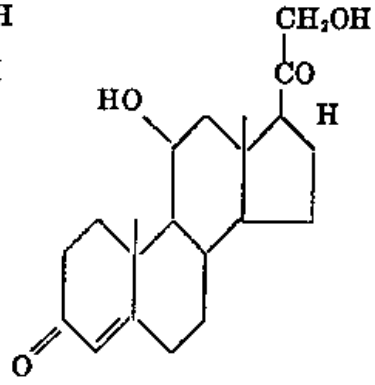
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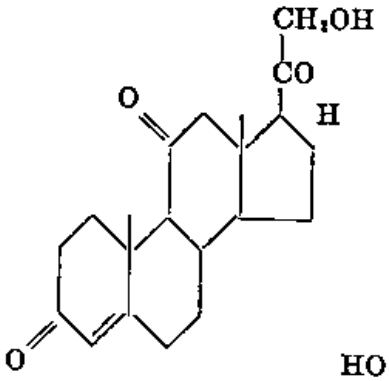
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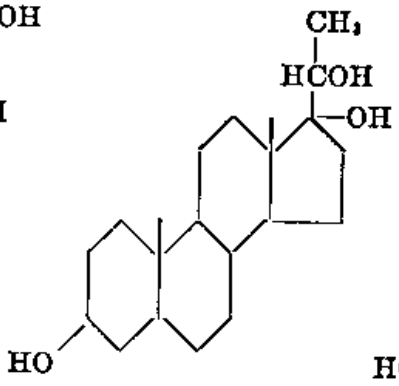
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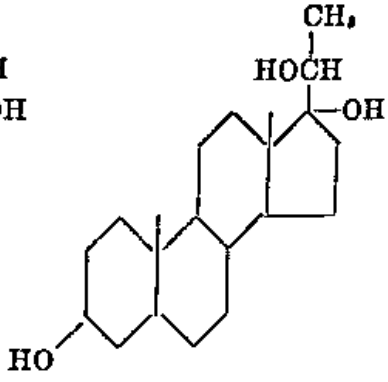
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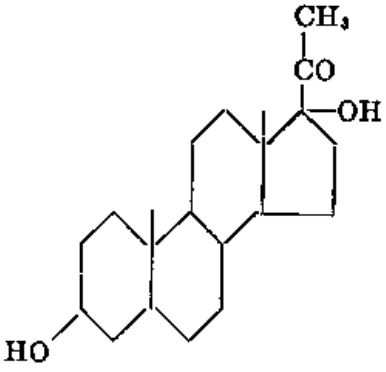
XVI



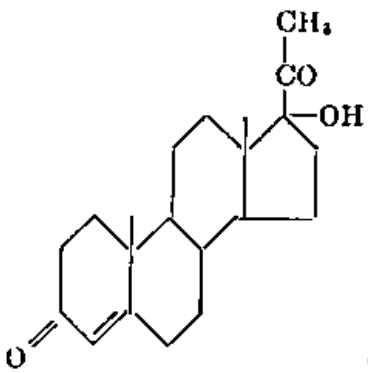
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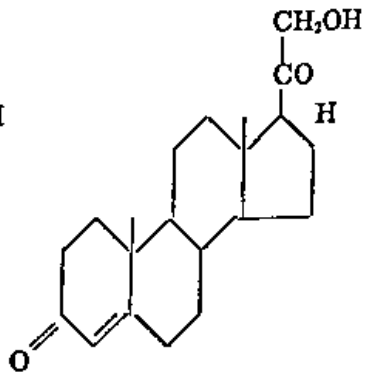
XIX



XX

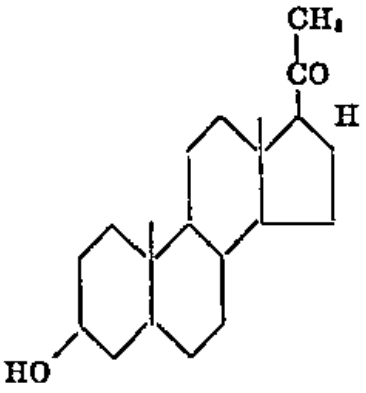


XXI

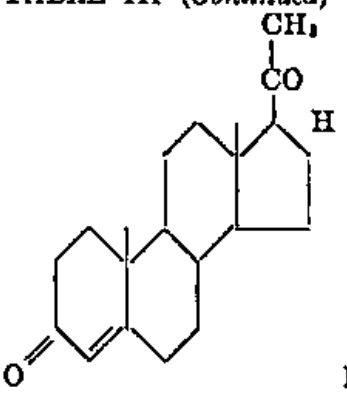


XXII

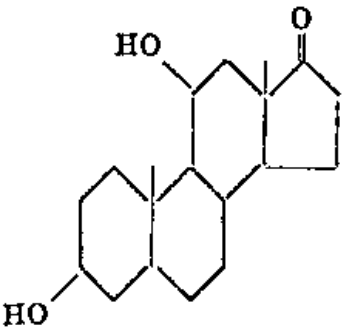
TABLE III (*Continued*)



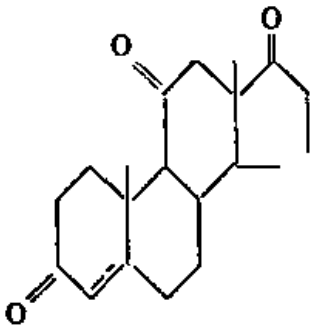
XXIII



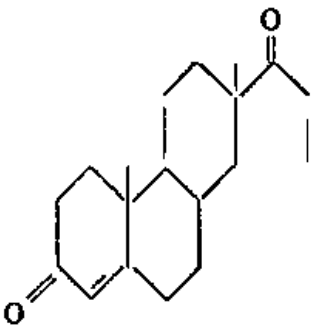
XXIV



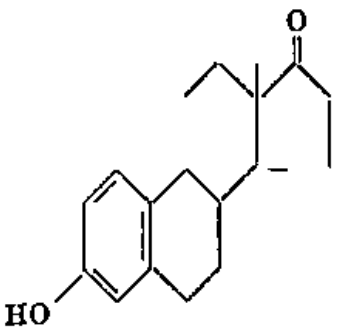
XXV



XXVI



XXVII



XXVIII

XII–XVI, and XXV–XXVI) Physiologically, the distinction is important since high gluconeogenic activity is observed only in the latter group, while marked action with respect to salt and water metabolism is found only in the desoxy series (*c*) Based on the substitution at C-17, the C_{21} compounds fall into a 17-hydroxylated group (Compounds I–XI and XVIII–XXI) and a group in which the fourth valency bond of C-17 is satisfied with hydrogen (Compounds XII–XVI and XXII–XXIV) Since the former give rise to the corresponding 17-ketosteroids by relatively mild oxidative procedures (Section III, C,2), the possible significance of the members of this group as precursors of the urinary 17-ketosteroids is of metabolic interest (see Section VII, A,1) (*d*) The compounds are divisible into an $\alpha\beta$ unsaturated 3-ketosteroid group (Compounds V–VIII, XI, XIV–XVI, XXI–XXII, XXIV, XXVI, and XXVII) and those in which ring A is saturated (Compounds I–IV, IX–X, XIX–III, XVIII–XX, XXIII, and XXV) The first mentioned resonant system is requisite to appreciable cortical activity (*e*) The presence in the side chain of a primary hydroxyl group vicinal to a carbonyl oxygen atom (*i e*, the primary α ketol grouping) differentiates these compounds (II–IV, VII–VIII, X–XIII, XV–XVI, and XXII), which are strongly reducing, from the remainder, which are not (see Section III, C,2) High cortical activity is confined to the first-mentioned class

There is species variation in the relative abundance of the various compounds in adrenal tissue Kuizenga (120) has summarized (Table IV) the quantities of the four principal carbohydrate-active hormones which may be separated from beef, hog, and sheep glands

TABLE IV

SPECIES VARIATION IN YIELDS OF 11 OXYGENATED ADRENAL HORMONES

Compound	Yield[a]		
	Ox	Hog	Sheep
Corticosterone (XV)	200		
Crude crystallizate[b]	700	1500	680
Dehydrocorticosterone (XVI)	150		
11 Dehydro-17 hydroxycorticosterone (VIII)	200	220	400
Crude crystallizate[c]		1700	850
17 Hydroxycorticosterone (VII)	75	610	0

[a] Mg /1000 lb of whole adrenal gland (data of Kuizenga, 120)

[b] According to assays consisting chiefly of corticosterone and dehydrocorticosterone

[c] According to assays, consisting chiefly of 11-dehydro-17 hydroxycorticosterone and 17 hydroxycorticosterone

B METHODS OF ISOLATION

A comprehensive description of the chemical procedures requisite to the isolation of the individual cortical steroids listed above would be superfluous In general, advantage is taken of (*a*) the observations of Pfiffner and Vars (184) and of Mason, Myers, and Kendall (166) that certain of the more highly oxygenated $C_{21}O_5$ and $C_{21}O_4$ compounds tend to pass from ether or benzene to water on repeated extraction, (*b*) the elegant method of Girard and Sandulesco (75) of separating water-insoluble, lipide-soluble ketones as their water-soluble, lipide-insoluble trimethylaminoacethydrazone chlorides which permits fractionation of a ketonic mixture according to the ease with which the carbonyl groups in various positions react to form these Girard complexes, and according to the hydrogen ion concentration required to hydrolyze the formed complexes, and (*c*) the application of the principles of chromatography, a method extensively employed by Reichstein, in which a mixture is adsorbed on a column of activated alumina and the components are eluted therefrom in order of their power of adsorption by the percolation through the column of appropriate organic solvent mixtures

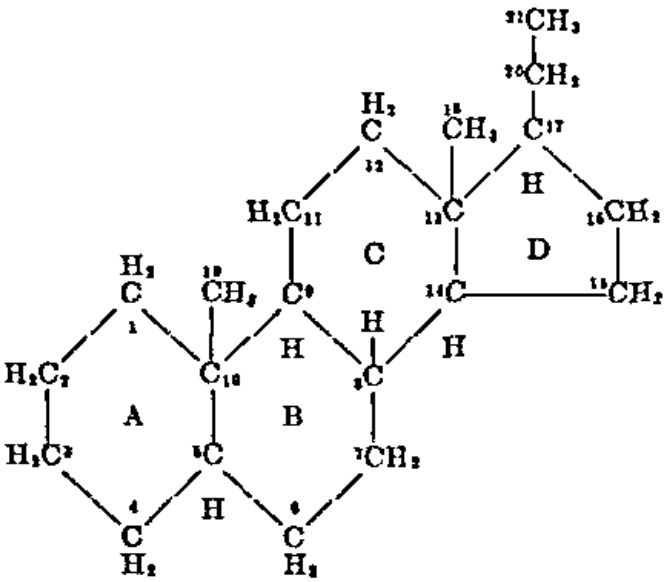
C STRUCTURE AND PROPERTIES

In this section only the established structure and principal chemical properties of the adrenal steroids are dealt with The many degradation

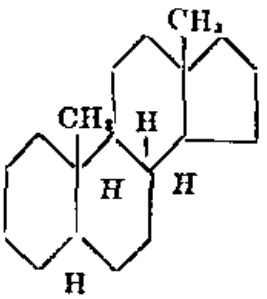
reactions, transformations from one series to another, and partial syntheses of inactive compounds which led to the elucidation of the structure of each individual are omitted, these are synopsized in the recent reviews on the chemistry of the adrenal steroids by Kuizenga (120), Reichstein and Shoppee (212), and Spring (241), in which references to earlier reviews and the original literature are listed in full

1 *The Carbon Ring Skeleton*

The parent saturated hydrocarbons of the adrenal steroids are allopregnane and androstane, the stereochemical formulation and system of numeration of which are given below



XXIX
Allopregnane



XXX
Androstane

Consistent with the burden of evidence the ring structures are formulated with the substituents of each pair of adjacent asymmetric carbon atoms (*i e* 5 10 9 10 8 9 8 14 and 13 14) oriented in space in the opposite or *trans* relation to each other Thus the two angular methyl groups at C-10 and C-13 lie on the same side (arbitrarily the near side) of the flat plane of the molecule and serve as points of reference

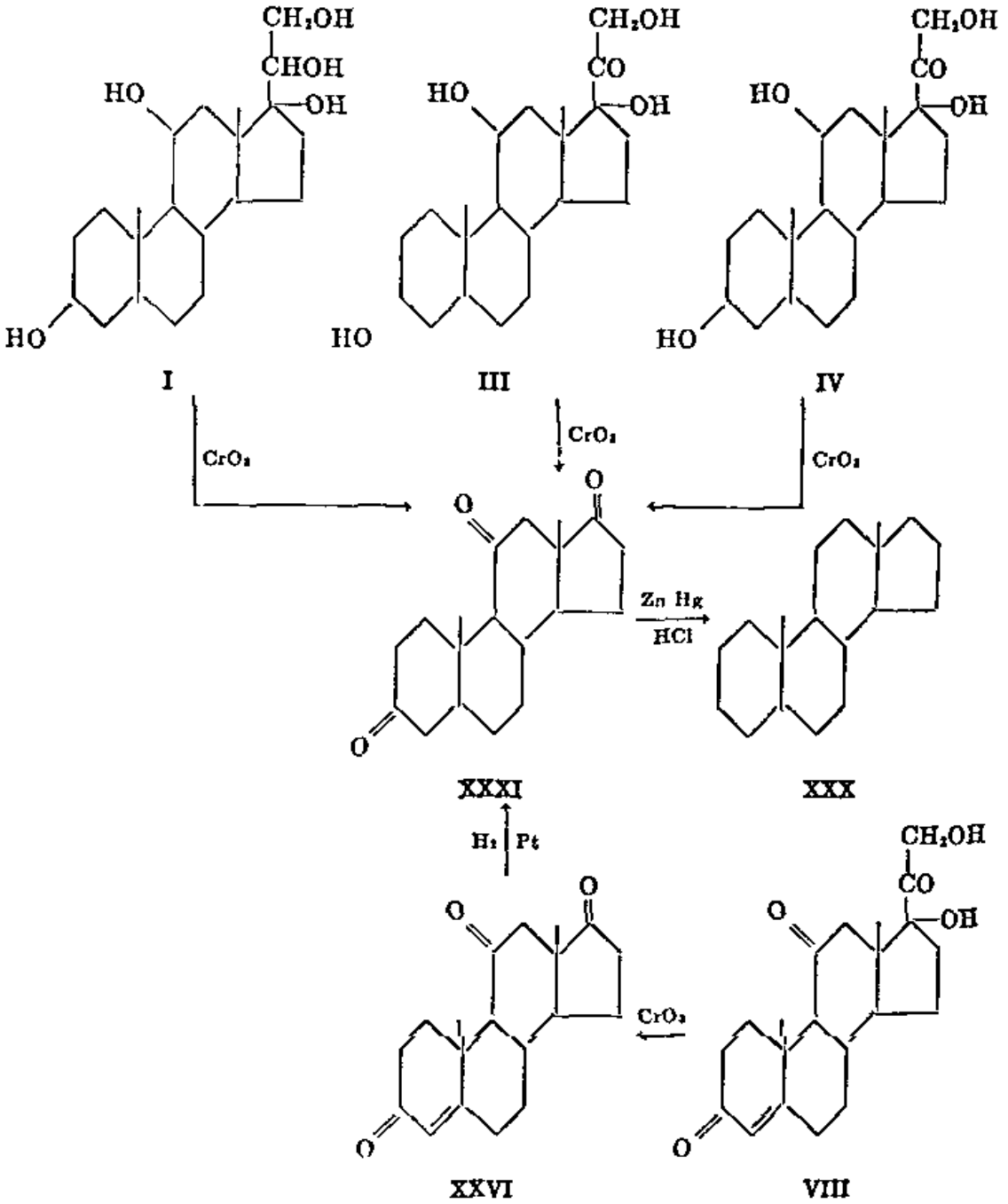
The spatial position of hydroxyl groups substituted in the nucleus is designated β or α This nomenclature was originally introduced by Fieser (56) to define the configuration of the 3-OH group (β) indicates the orientation with respect to the remainder of the molecule that obtains in cholesterol and β-cholestanol and (α), the reverse stereochemical relationship formulated respectively, by a solid and a dotted valency bond line The convention has been universally adopted and extended to define the configuration of hydroxyl groups at centers of asymmetry other than C-3 (see also page 564) Except in the *lumi* series (page 565) (β) implies that the hydroxyl group is located on the same side of the flat plane of the molecule as the angular methyl groups at C-10 and C-13 and (α) indicates *trans* orientation with respect to these reference groups

The observation which led to the establishment of the steroid ring skeleton of the adrenal compounds was provided by Reichstein (202)

who noted the high androgenic potency of Compound G of his series, adrenosterone (XXVI), which immediately suggested a close chemical relationship to the male sex hormones Also the biologically inactive

TABLE V

PROOF OF THE RING SKELETON OF THE ADRENAL STEROIDS

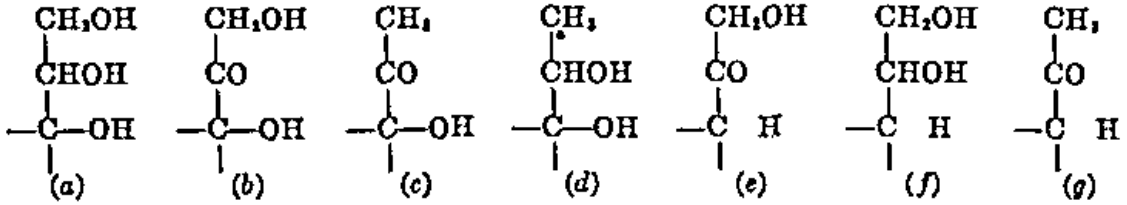


compounds A C, and D (I, III and IV), on treatment with chromic anhydride (Table V) yielded the saturated triketone XXXI with androgenic activity (203) and identical with the product of reduction of the

double bond of adrenosterone (XXVI) At about the same time, Mason Myers, and Kendall (167) obtained, from the chromic acid oxidation of their cortically active Compound E (VIII), an androgenic product which proved to be adrenosterone (XXVI) Unequivocal chemical proof of the carbon ring structure came with the Clemmensen reduction of XXXI to the known parent hydrocarbon, androstane (XXX) (Reichstein, 204) Later Steiger and Reichstein (244) degraded corticosterone (XV) to allopregnane (XXIX)

2 *The Side Chain*

Seven types of two-carbon side chains at C-17 are characteristic of the adrenal steroids



The presence of a ketonic oxygen atom at C 20 adjacent to the primary hydroxyl group at C 21 (*i e* the α ketol grouping, types *b* and *e*) is essential to cortical activity and imparts to the molecule reducing properties similar to those of fructose which contains the same α-ketol grouping Only primary α-ketols of types *b* and *e* reduce alkaline silver diamine, also they reduce cupric ion in alkaline solution and phosphomolybdic acid, reactions which have been applied to the quantitative estimation of small quantities of reducing steroids of these types (Sect VII, A,3)

The behavior of the various types on oxidation with excess chromic or periodic acid is characteristic and has been extensively employed in the elucidation of the substituents at C-17, C-20 and C-21 With CrO_3 the 17-hydroxylated compounds (types *a* to *d*) are oxidized to the corresponding 17-ketosteroids, $-\overset{|}{\underset{|}{C}}=O$, and the C-17 unsubstituted types *e* and *f*, to the corresponding *etio* acids, $-\overset{|}{\underset{|}{C}}(COOH)-H$ With periodic acid, the 17 hydroxylated α glycols (types *a* and *d*) give the corresponding 17-ketones, while, from the dihydroxyacetone type *b*, the 17-hydroxy *etio* acids, $-\overset{|}{\underset{|}{C}}(COOH)-OH$, are obtained, and from the C-20–C-21 glycols *f*, the 17 aldehydo compounds, $-\overset{|}{\underset{|}{C}}(CHO)-H$

Owing to the asymmetry about C-17 in the allopregnane series, the hydrogen atom or hydroxyl group substituted at the junction of the side chain may be oriented in either the *cis* (β) or the *trans* (α) position with respect to the rest of the molecule, conversely the alkyl side chain must occupy the opposite spatial position Since the sterols may be degraded (under conditions which tend to preclude inversion at C-17) to the naturally occurring bile acids and members of the C-21 hormone series not hydroxylated at C-17, it follows that the configuration of the side chain is the same in all three classes of steroids Recent physical and chemical evidence (see page 574) makes it practically certain that the angular methyl group at C-13 and the side chain at C-17 are oriented on the same side of the flat plane of the molecule, accordingly the side chain is now assigned the 17(β) configuration, *i e*, the opposite to that provisionally accepted prior to 1946

17(β) and 17(α) here refer to the orientation of the side chain, the 17 hydrogen atom occupies conversely the α and β positions As yet there is no universally accepted nomenclature to distinguish the natural from the artificial series of 17 hydrogen steroids In the 17 hydroxylated series, however, Reichstein and Gatzi (210) define the configuration about C-17 by stating that of the 17 hydroxyl group and in 1938 they arbitrarily assigned to the natural compounds the 17(β) OH configuration (see the text below) Selye (227), however, prefers a system of nomenclature in which the orientation and constitution of the alkyl side chain are specifically defined in relation to, and as a substituent of, the basic ring structure

In an effort to reach a generally applicable and uniform system of nomenclature which permits convenient designation of the orientation about all centers of asymmetry in the steroid molecule, the author and D A Prins offer the following suggestions

(*1*) That such a system should be based upon the accepted stereochemical structure of the ring systems of androstane (XXX) and etiocholane on the one hand and of allopregnane (XXIX) and pregnane on the other Since the members of each pair differ only in the orientation of the hydrogen atom substituted at C-5, the long established use of the prefix *allo* is thus retained *exclusively* for the designation of the C-5 configuration of the parent hydrocarbons of all classes of steroids

With respect to the inversion of a hydrogen atom, or a methyl group, about all other centers of asymmetry (*i e*, carbon atoms 8 9, 10, 13, 14, and 17 of the ring skeleton and 20 24 *etc* of an aliphatic side chain), it is suggested that the prefix *iso* (not abbreviated) should be employed (together with the numbers of the carbon atoms concerned) to designate the configuration opposite to that which obtains in androstane (or etiocholane), allopregnane (or pregnane), allocholane (or cholane) cholestane (or coprostane), *etc* This nomenclature merely represents an extension of the use of *iso* in the sense originally introduced by Butenandt and adopted by Reichstein and others *i* (an abbreviation of *iso*) is thus retained for the designation of condensed five ring systems containing a 3,5 bridge (*i e*, members of the *i* androstane, *i*-cholestane series, *etc*)

(*2*) That the *established* configuration of functional groups substituted in the nucleus should be designated as (α) or (β) (see page 561), *the indices being placed in parentheses* (see Callow 27 and Shoppee 231) Where doubt exists concerning the stereochemical orientation it is suggested that the indices be contained in inverted commas rather than brackets (see Petrow and Starling, 183), *e g*, cholestane-3(β)-

7 α -diol (151) and cholestane 3(β) 7 'β diol (267) in each of which the orientation of the 7 hydroxyl group is still in doubt (188) In instances of complete uncertainty of configuration *epi* may serve to differentiate the members of a pair (see page 574), further, to avoid confusion it would seem desirable to restrict the use of *epi* with reference to functional groups only

As regards tertiary hydroxyl groups Ruzicka and Muhi (217) have introduced a system of nomenclature adopted by others (189 193 211) in which the orientation of the 5 hydroxyl is defined in terms of the parent hydrocarbon *e g* cholestan 5-ol[5(α) OH] and coprostan 5-ol[5(β) OH] This system may well be extended to include all functional groups and to bear on all tertiary positions in the molecule thus, '3β 21-diacetoxy 14-oxy 20-keto 17 iso 5 14-diallo pregnan (191) would be expressed 14 *iso* 17 *iso* allopregnane 3(β) 14 21 triol 20-one 3 21 diacetate

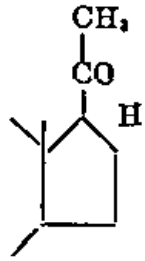
The isomerism of hydroxyl and other functional groups substituted in an aliphatic side chain is of the diastereoisomeric type consequently absolute configurations cannot be assigned The empirical use of α and β in this connection as introduced by Marker in the 17 hydrogen series and the arbitrary assignments of Prins and Reichstein (195 196) in the 17 hydroxyl series thus need to be retained until correlations are established Omission of the brackets serves to distinguish diastereo isomerism of this type from *cis trans* isomerism (see Shoppee 231) *e g* pregnane 3(α) 20α-diol

The orientation of alkyl substituents of tertiary carbon atoms may be expressed in terms of the parent hydrocarbon the configuration of those attached to nuclear methylene groups or in the side chain may be designated normal and *iso*

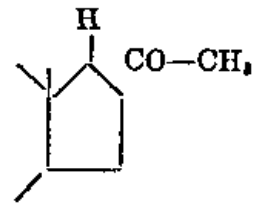
In the main the above proposals conform with general practise but the terms *allo epi* and *iso* are not infrequently used indiscriminately with reference to a hydrogen atom a hydroxyl group or a methyl group For example to denote the reverse configuration of the 14 hydrogen atom Hirschmann and Wintersteiner (101) employed *epi* (14-*epi*-equilenin) while Bachmann *et al* (2) describe the same compound as *iso*equilenin and in the cardiac aglucone series inversion of the 14 H atom is indicated by 14-*allo* (Plattner *et al* 191) and also by 14-*epi* [Meyer (168)] Similarly the prefix *lumi* has been used to designate inversion of both the methyl group at C-10 (*i e* lumisterol) and that at C-13—*i e* *lumi* androsterone (21) and *lumi* estrone (20) according to the nomenclature herewith suggested these compounds would be more specifically defined respectively as 10 *iso* ergosterol 13-*iso*-androsterone and 13 *iso* estrone The two artificial C_7 isomers of etiodesoxycholic acid (formulas 3 and 4 page 575) are thus 3(α) 12(α)-dihydroxy 17 *iso*-etiocholanic acid and 3(α) 12(β) dihydroxy 17-*iso* etiocholanic acid Likewise reversal of the natural configuration about C-20 has been observed on several occasions—*e g* 3(α) 12(α) dihydroxy 20 *iso*-bisnorcholanic acid (238 239)—and instances of inversion about more than one center of asymmetry are not uncommon—*e g* pyrocalciferol (9 *iso*-10-*iso*-ergosterol) 3βoxy 17 *iso* 5 14-dialloatiocholansaure (191) [3(β)hydroxy 14 *iso*-17 *iso* etioallocholanic acid] As in the designation of functional groups of uncertain orientation inverted commas may profitably be employed when the site of inversion is not conclusively established (*e g* 9 *iso* pregnane (urane) see page 618)

Several methyl ketones (type *g*) are known in both isomeric forms which are reversibly interconvertible by the action of acid or alkali (17,18 23) The stable 17(β) isomer predominates in equilibrium mixtures and is that which arises directly from the sterols on oxidation, the artificial 17(α) isomer is labile and physiologically inactive In formulation, the side chain of the natural 17(β) series conventionally occupies

the vertical position, and that of the artificial 17(α) series, the horizontal position, as illustrated below

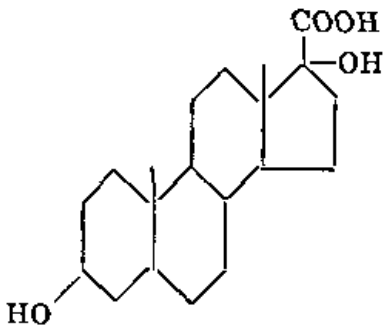


Stable 17(β) series

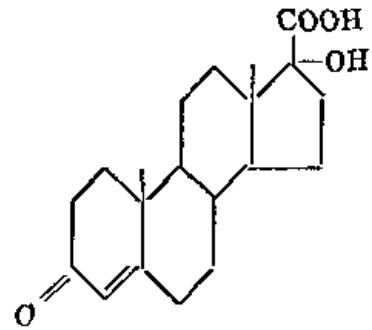


Labile 17(α) series

The stereochemical relationship of the side chain in those steroids hydroxylated at C 17 is difficult to establish since removal of the hydroxyl group destroys the asymmetry about C-17 However, strict chemical proof that all members of the 11-desoxy-17-hydroxy adrenal series (Compounds IX–XI and XVIII–XXI), types *a* to *d*, are similarly oriented at C-17 has been furnished by Reichstein and collaborators by interconversion and degradation reactions leading to the same 3(β),17"β"-dihydroxyetioallocholanic acid (XXXII) or 17"β"-hydroxy-3 keto Δ^4 etiocholenic acid (XXXIII) Both C_{17} epimers of XXXII and



XXXII



XXXIII

of XXXIII have been prepared artificially and are not identical with the breakdown products of the natural substances In the case of the degradation of the 11-oxy 17-hydroxy compounds (I–VIII, types *a* to *d*), the corresponding 17-epimeric pairs of reference acids are not characterized, but the application of optical superimposition rules defining the contribution of the 11-hydroxyl or 11-ketone group to the molecular rotation leaves little doubt of the same configuration at C 17 in these instances In 1938, when the side chain of the sterols and bile acids was believed to be *trans* oriented, Reichstein and Gatzi (210) arbitrarily assigned the β configuration to the 17-hydroxyl group of the natural adrenal series, and they are so described in the literature to date, this would orient the side chain of the 17 hydroxylated adrenal compounds as in the sterols and bile acids, which seems highly likely With the

evidence now overwhelmingly in favor of the *cis* position of the side chain in the 17-hydrogen compounds (see page 574), reversal of the configuration previously assigned in the 17 hydroxy series is indicated In their latest communications, Reichstein and collaborators (53,240) point to the probability that the so-called 17"β"-OH compounds are in reality 17(α) Because of this uncertainty and the fact that revision has not yet been effected, the original assignment of Reichstein and Gatzi (210) is retained throughout this review, with the use of inverted commas to indicate its arbitrary nature (see page 564), in formulation however, the natural series (probably 17(α) OH) is indicated by a vertical side chain, and the artificial epimers (probably 17(β)-OH), by a horizontal side chain

Isomerism about the center of asymmetry at C 20 is possible in types *a*, *d*, and *f*, and a naturally occurring pair is observed in Reichstein's Compounds J (XVIII) and O (XIX), in which the 20β and 20α configurations, respectively, are arbitrarily assigned without commitment as to absolute spacial orientation (see Prins and Reichstein 196, and page 564)

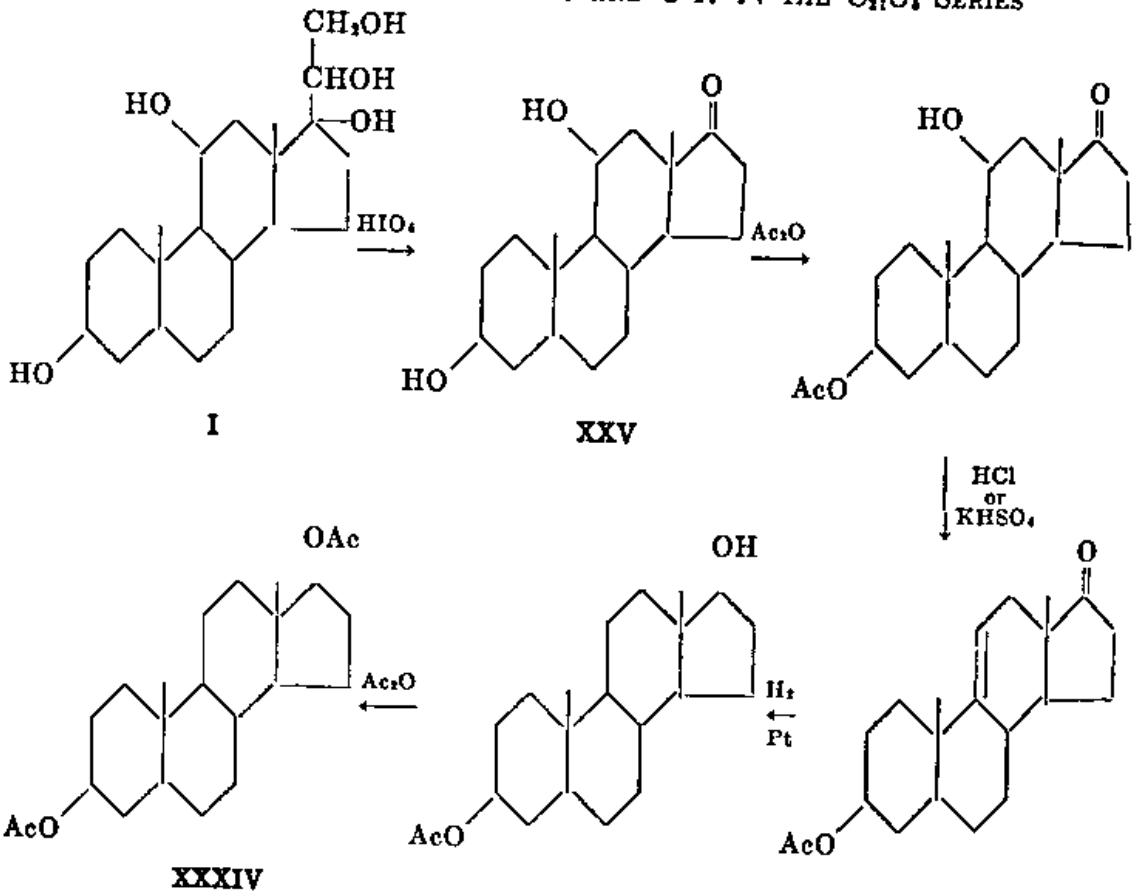
3 *The Nuclear Substituents*

a Position In the adrenal steroids not oxygenated at C 11 (the 11-desoxy series), the position and nature of the nuclear substituents were readily ascertained by degradation to known compounds On oxidative cleavage of the side chain with chromic acid, the secondary alcohol groups are simultaneously oxidized to ketones, and the resulting product is one of (*a*) androstane-3 17-dione, (*b*) Δ^4 androstene-3 17-dione, (*c*) 3 keto-etioallocholanic acid, or (*d*) 3 keto Δ^4 etiocholenic acid With periodic acid, the nuclear hydroxyl groups remain unattacked, and, depending on the type of side chain (see Sect III C 2) one or two carbon atoms are removed to give (*a*) androstan-3(β)-ol-17 one, (*b*) Δ^4 androstene-3,17-dione, (*c*) 3(β),17"β"-dihydroxyetioallocholanic acid (XXXII), or (*d*) 17"β"-hydroxy-3 keto-Δ^4-etiocholenic acid (XXXIII)

The substituent at C-11 in the remaining members of the series proved much more difficult to place, as at the time of the investigations no authentic 11-oxygenated steroid was available for comparison As previously indicated (Table V, page 562), the unsaturated $C_{21}O_5$ compounds (V–VIII), give with chromic acid adrenosterone (XXVI), which, on saturation of the double bond, yields the same androstanetrione (XXXI) as that formed on oxidative cleavage of the side chain of all saturated members of the $C_{21}O_5$ series (Compounds I–IV) The position of the nuclear substituents in Compounds I–VIII is thus the same in all cases Dehydration (Table VI) of the 11-hydroxyl group

TABLE VI

PROOF OF SUBSTITUTION AT C 3 AND C 17 IN THE $C_{21}O_5$ SERIES

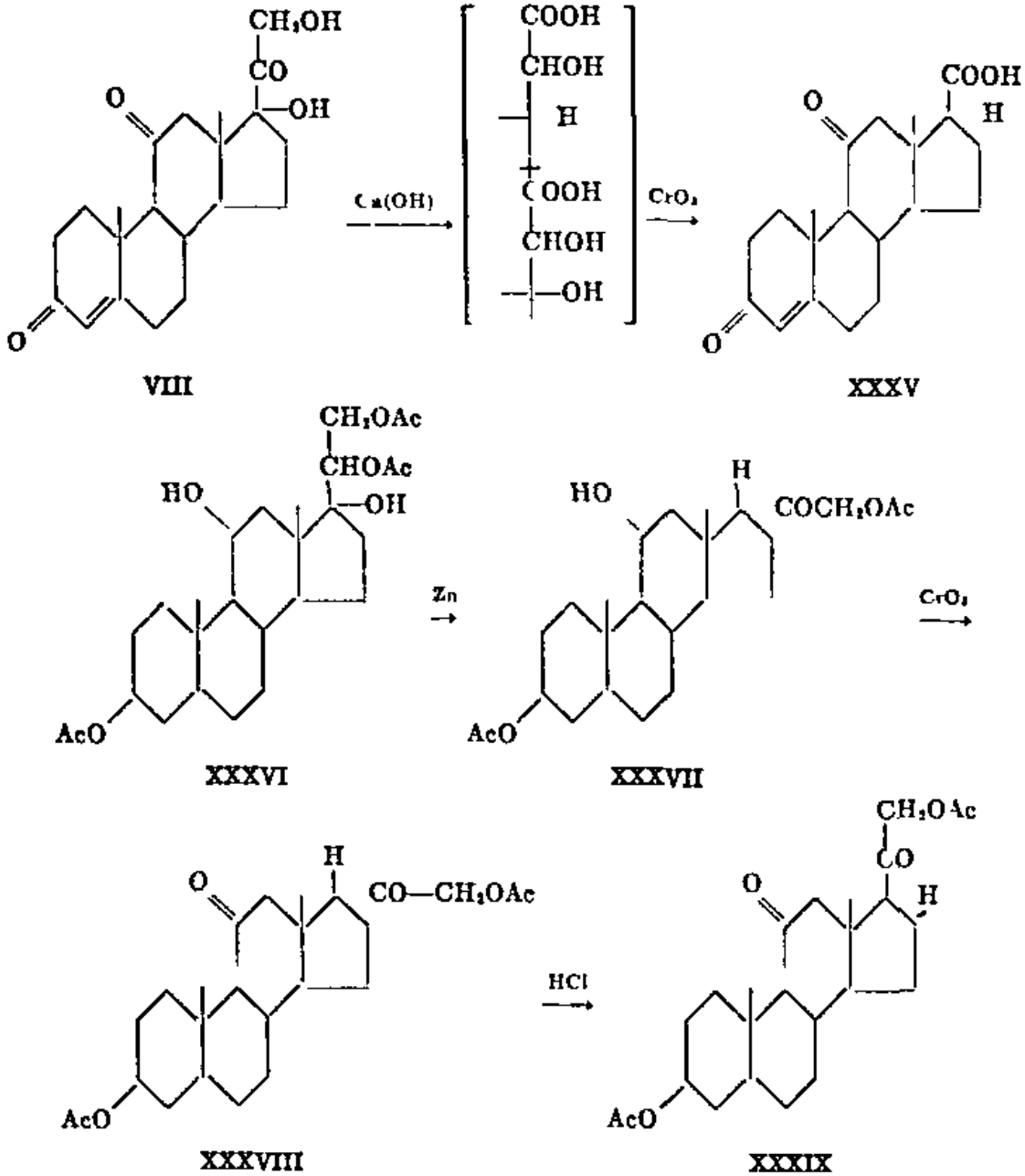


of the ketone XXV obtained on cleavage of the side chain of Reichstein's Compound A (I) and subsequent catalytic hydrogenation and acetylation led to the known androstane-3(β)-17(α)-diol diacetate (XXXIV), which established the presence of the 3(β)-OH group in I and of carbonyl oxygen atoms at C-3 and C-17 in the triketone XXXI (229) The third ketone group of the latter exhibited unique chemical properties in that it failed to react in the usual manner to form an oxime, semicarbazone, *etc* The hindered 11 position was assigned to this substituent by Steiger and Reichstein (242) in 1937 by a process of elimination Positions 1, 2, 4, 15, and 16 were excluded, as the products would behave as an α or β diketone, which was not in accordance with fact, and a carbonyl group in position 6, 7, or 12 would not be expected to be unduly unreactive That the $C_{21}O_4$ corticosterone group (Compounds XII–XVI) also contain an 11-oxygen was shown by the elimination of the 17-hydroxyl group of the $C_{21}O_5$ compounds to products identical with or referable to the $C_{21}O_4$ substances (Table VII) On treatment of Kendall's compound E (VIII) with calcium hydroxide and oxidation with chromic acid, Mason (159) obtained small yields of 3,11-diketo Δ^4-etio-

cholenic acid (XXXV) identical with the product of oxidation of corticosterone and dehydrocorticosterone (XV and XVI) From the triacetate (XXXVI) of their Compound A (I), Shoppee and Reichstein

TABLE VII

CORRELATION OF THE $C_{21}O_4$ AND $C_{21}O_4$ SERIES



(232) effected with zinc the elimination of acetic acid to a substance XXXVII, isomeric at C-17 with Compound R (XII), which, on oxidation, yielded the diketone XXXVIII, rearrangement in acid of the labile side chain of the latter to the stable configuration gave the

diacetate (XXXIX), of Compound N (XIII) Full confirmation of the assignment at C-11 came with the artificial preparation (Sect IV B) of 3 11-diketo Δ^4-etiocholenic acid (XXXV) from desoxycholic acid (126)

b Stereosomerism of the Hydroxyl Groups The hydroxyl group at C 3 is *cis*(β) oriented in all of the natural adrenal steroids with the exception of Compound III (53), thus Compounds I, II, IV, IX, X, XII, XIII, XVIII–XX, XXIII, and XXV form insoluble adducts with digitonin

As regards the orientation of the 11-hydroxyl group in Compounds I, II, III, V, VII, XII, XV, and XXV, the interconversion and correlation reactions formerly establish the same spatial relationship in all instances, but the assignment of the 11(β) configuration has only recently received full confirmation through the synthetic approach from desoxycholic acid (LII) (see Sect IV B, and reference 53) In the bile acid series, several pairs of 11 hydroxy epimers have been prepared artificially, one epimer is esterifiable and resists dehydration on treatment with acid while, in the opposite configuration, the alcohol is difficultly esterified, if at all, and loss of the elements of water proceeds easily in an acid medium to a mixture of the corresponding $\Delta^{9\,11}$ and $\Delta^{11\,12}$ derivatives (see Table VI, page 568) Reference to three dimensional models makes it apparent that the *cis*(β)-oriented 11 hydroxyl group is subjected to considerable steric hindrance from the angular methyl groups neighboring at C 10 and C-13 and lying on the same side of the flat plane of the molecule, with the 11(α) hydroxyl group on the opposite side of the molecule to the angular methyl groups, interference is diminished and substitution may be expected Accordingly the non-reactive, readily dehydratable 11-hydroxyl of the corticosteroids is assigned the β configuration

IV Artificial Preparation of the Active Adrenal Steroids

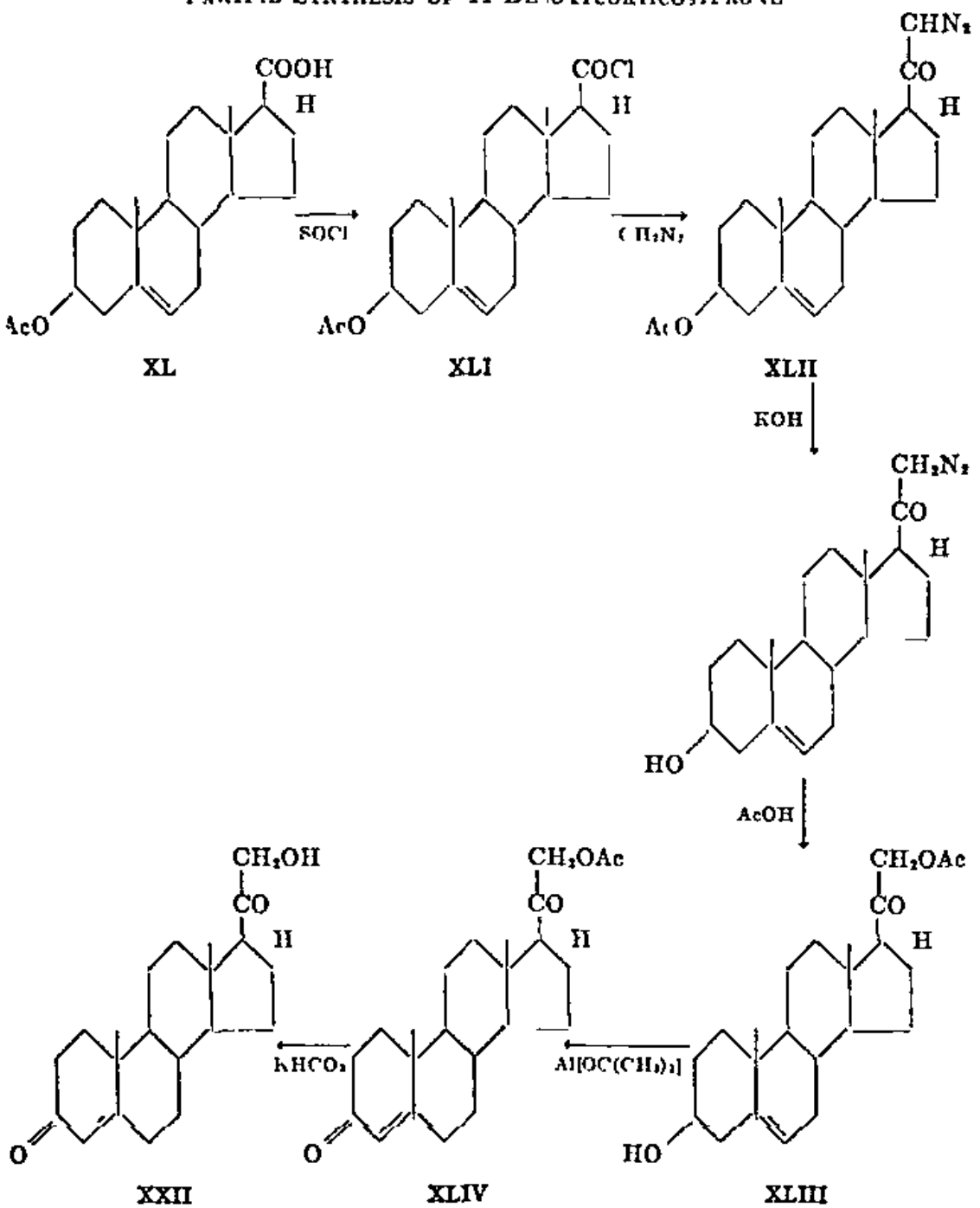
A The 11-Desoxy Compounds

1 *Partial Synthesis of 11 Desoxycorticosterone*

In 1937 Steiger and Reichstein (243) prepared desoxycorticosterone acetate (XLIV) from 3(β) acetoxy Δ^5-etiocholenic acid (XL), and showed that this hitherto unknown product was highly active in the Everse-de Fremery test This conversion, while it marks the first preparation by artificial means of a naturally occurring biologically active adrenal steroid, antedates by approximately one year the isolation of desoxycorticosterone from adrenal tissue (Reichstein and von Euw, 209) The process (Table VIII) is widely used in the commercial preparation of desoxycorticosterone The immediate starting material in the partial

TABLE VIII

PARTIAL SYNTHESIS OF 11 DESOXYCORTICOSTERONE



XL XLI XLII

XXII XLIV XLIII

synthesis,[8] 3(β)-acetoxy-Δ⁵ etiocholenic acid (XL) is readily available as a by product in the chromic acid oxidation of cholesterol to dehydro-*iso*androsterone (XLV) Addition of diazomethane to the acetoxy acid

[8] In that the starting materials of all preparations are of natural origin (the sterols and bile acids), the term "partial synthesis" is applied as distinct from "total synthesis," which implies that the products may be built up from the constituent elements

chloride XLI gives the acetoxy diazo ketone XLII, which is saponified and hydrolyzed with acetic acid to 21-acetoxypregnenolone (XLIII) Desoxycorticosterone acetate (XLIV) is then furnished by the oxidation of the 3-hydroxyl group, either with aluminum alkoxide by the method of Oppenauer, or by chromium trioxide after previous saturation of the double bond with bromine and subsequent debromination, in the course of either oxidative procedure the 5,6 ethylenic linkage migrates to the 4,5 position in conjugation with carbonyl oxygen atom at C-3

2 *Partial Synthesis of 17-Hydroxy-11 Desoxycorticosterone*

More difficulty attended the partial synthesis (Table IX) of 17-hydroxy 11-desoxycorticosterone (XI), and the compound is not yet generally available From dehydroisoandrosterone (XLV), von Euw and Reichstein (51) prepared the tetrol XLVIII by the method of Butenandt and Peters (19), a Grignard reaction with allyl magnesium bromide and an Oppenauer oxidation lead to 17"α"-allyltestosterone (XLVI), which, on dehydration of the tertiary alcohol, gives the triene XLVII, the two side chain double bonds of which are then hydroxylated with osmium

TABLE IX

PARTIAL SYNTHESIS OF 17 HYDROXY 11 DESOXYCORTICOSTERONE

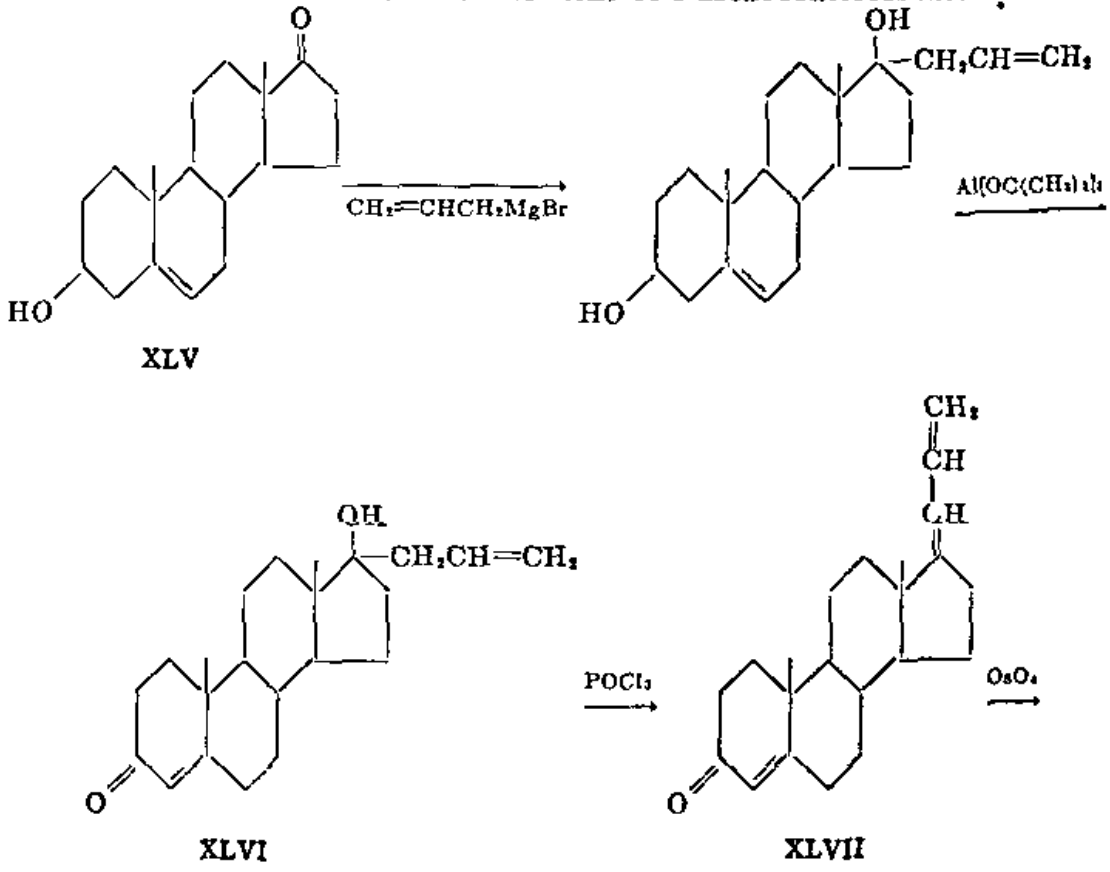
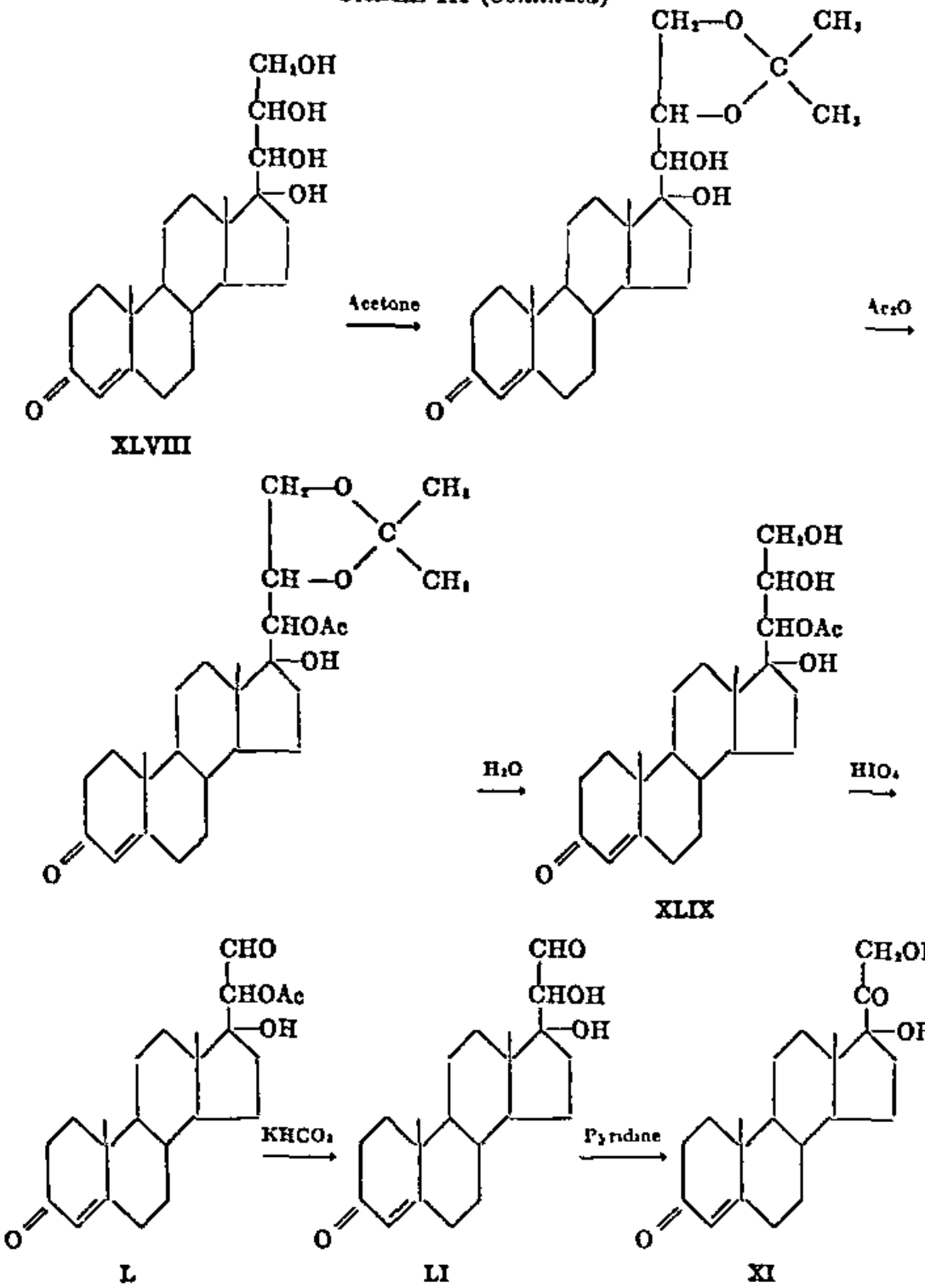


TABLE IX (*Continued*)



tetroxide Following protection of the 21- and 22-hydroxyl groups with acetone, the C_{20} monoacetate XLIX is obtained on acetylation and hydrolysis of the acetal Oxidation of this glycol with periodic acid yields the acetoxy aldehyde L. The free dihydroxy aldehyde LI,

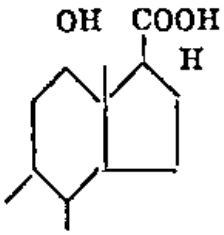
obtained on gentle saponification of L, rearranges in hot pyridine to the dihydroxyacetone XI The overall yield of 17-hydroxy-11-desoxy-corticosterone (XI) is extremely small

B The 11-Oxygenated Compounds

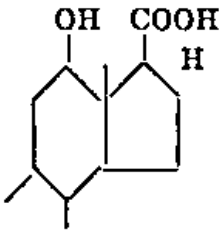
Because of the probable therapeutic value of adrenal steroids possessing carbohydrate activity, the preparation by artificial means of members of the O_{11} series has received special attention over the past six years 11 Dehydrocorticosterone (XVI) was first realized in minute yield in 1943 by Lardon and Reichstein (127) in Switzerland In America, the promising approaches to the same end by Kendall and others led to the establishment of a Conference on the Synthesis of E under the aegis of the Office of Scientific Research and Development The researches of the members of this group and their collaborators have not yet been published in full

Apart from the adrenal hormones themselves, 11-oxygenated steroids are not known to occur in nature Thus, starting material is demanded which will permit the introduction of a ketonic or alcoholic oxygen function at C-11 and of the α,β unsaturated 3-ketone grouping in ring A Desoxycholic acid—3(α),12(α)-dihydroxycholanic acid LII (see small type below)—admirably serves the purpose (*a*) it is available in large quantities from bile (*b*) the 12 substituted hydroxyl group provides inroad to ring C, and (*c*) the corresponding 3 keto derivatives are readily brominated at C-4 because of the *cis* configuration at C-5

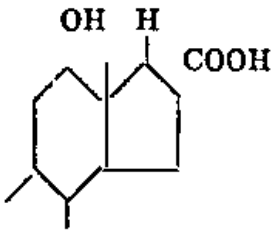
The spatial orientation of hydroxyl groups at C 11 and C-12 and of the side chain at C-17 has only recently been established Koechlin and Reichstein (119) in 1942 assigned to natural desoxycholic acid the structure 3(α) 12(β)-dihydroxycholanic acid the β configuration of the 12 hydroxyl group thus conformed with the deduction reached from X ray measurements by Giacomello (74) that the 12 hydroxyl group the methyl group substituted at C-13 and the C 17 side chain should all lie on the same side of the molecule Later (1944) Sorkin and Reichstein (238) observed that bisnordesoxycholic acid failed to lactonize, while 12-*epi*bisnordesoxycholic acid formed the cyclic structure and correctly concluded (contrary to the view held by Giacomello) that in the natural bile acid series the 12-hydroxyl group and the side chain must be *trans* to each other They retained the 12(β) OH configuration and assumed that the side chain must occupy the α position Meanwhile, Gallagher and Long (70) had accumulated convincing evidence that the reverse is true *i e*, that the 12 hydroxyl is *trans* oriented and the side chain *cis* oriented each with respect to the C-13 angular methyl group Further proof of the latter was provided by Sorkin and Reichstein (240) with the preparation of all four possible epimers about C-12 and C-17 of etiodesoxycholic acid (*1* to *4*, below) Reference to space models clearly indicates that facile lactonization can take place only when the hydroxyl and carboxyl



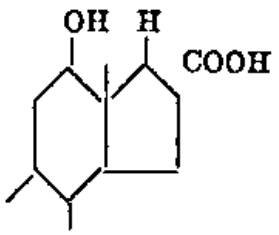
(*1*)



(*2*)



(*3*)

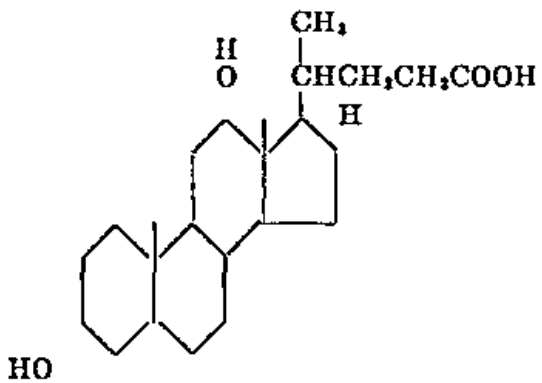


(*4*)

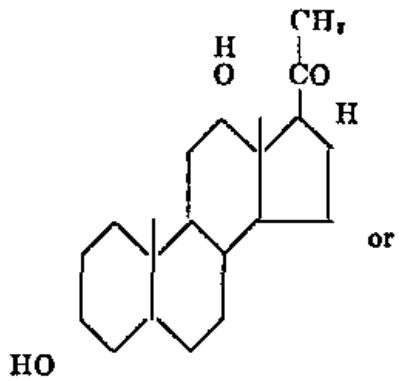
groups lie together on the opposite side of the molecule to the angular methyl group, conditions which are satisfied only by structure *3* Of the four acids only one formed a lactone, that derived from natural desoxycholic acid by the isomerization of the C-17 side chain accordingly it must be assigned structure *3* The extensive X ray studies of Crowfoot and collaborators (33) fully bear out the deductions reached from the chemical approach

The reactions requisite to the conversion of desoxycholic acid to adrenal steroids with carbohydrate activity fall into four discrete groups (*1*) degradation of the bile acid side chain to products (LIII, LIV and LV) suited to the building up of the α ketol side chain types *b* or *e*, (*2*) the transposition of oxygen from the 12 to the 11 position, (*3*) the building up of the α ketol or dihydroxy acetone type side chain, and (*4*) the introduction of the α,β unsaturated 3 ketone grouping in ring A

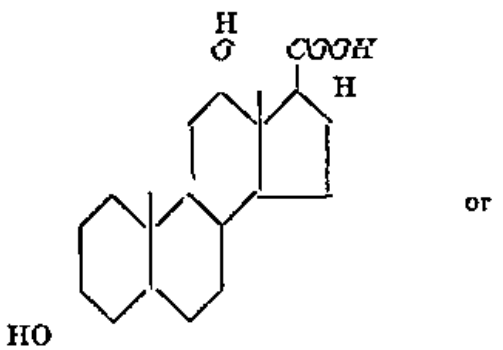
1 *Degradation of the Bile Acid Side Chain*



LII

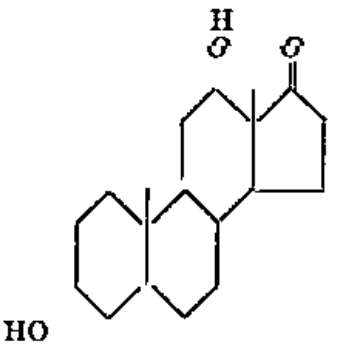


LIII



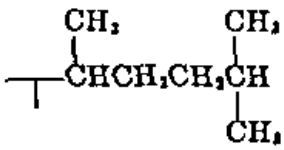
LIV

or



LV

Degradation of the side chain of desoxycholic acid (LII) to the corresponding methyl ketone LIII, etio acid LIV, or 17-ketone LV constitutes the first essential step of the partial syntheses, and it is through this stage of the entire procedure that the most unsatisfactory yields are encountered Unlike cholesterol, which, as acetate dibromide, may be oxidized directly with chromic acid to give (after debromination and saponification) moderate amounts of pregnenolone, 3(β)-hydroxy-Δ^5-etiocholenic acid, and dehydroisoandrosterone (XLV), the diacetate of methyl desoxycholate (LVI), oxidized under similar conditions, yields only traces (less than 0 1%) of the diacetates of LIII, LIV, and LV (30,88, 197,200) No worthwhile improvement is effected by the extension, prior to oxidation, of the bile acid side chain to the saturated aliphatic norsterol side chain (88)



The classical Barbier-Wieland stepwise degradation, first applied to desoxycholic acid by Hoehn and Mason (102) in 1938, affords the desired

TABLE X

STEPWISE BARBIER WIELAND DEGRADATION OF THE BILE ACID SIDE CHAIN

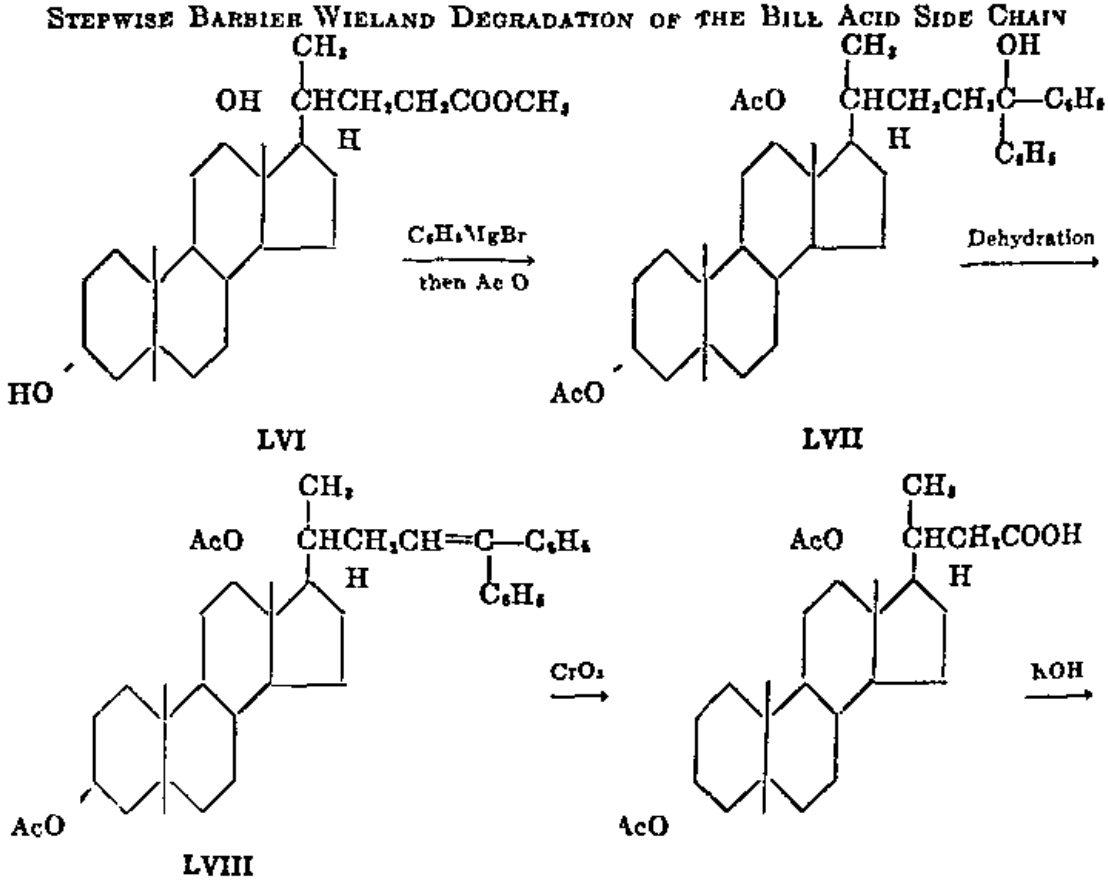
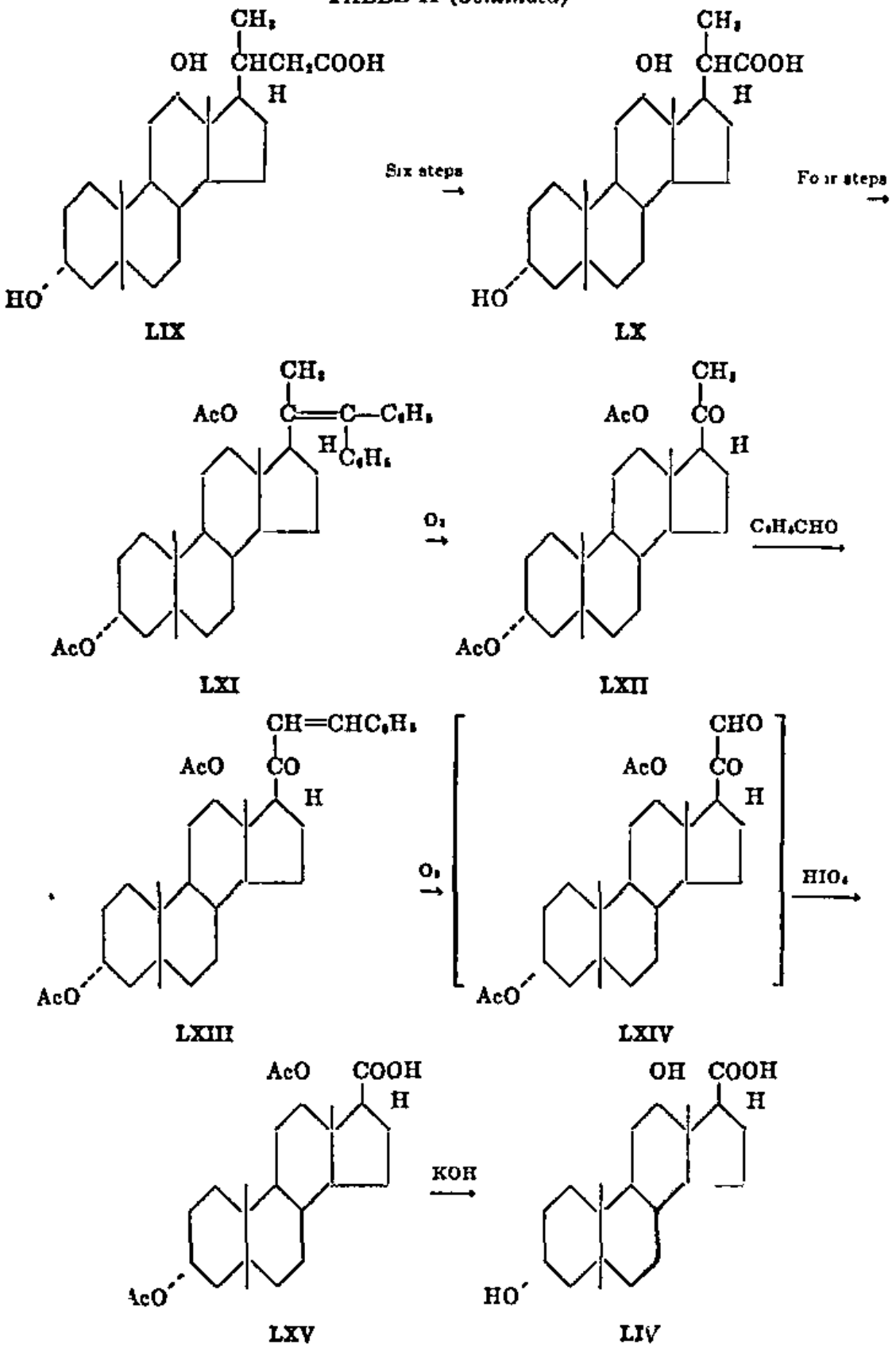


TABLE X (*Continued*)



products in 2–5% yield (Table X) The procedure may be carried out either before or after the introduction of the oxygen atom at C-11 (Sect IV B2)

Methyl desoxycholate (LVI), through a Grignard reaction with phenyl magnesium bromide and acetylation, is converted to the diphenylcarbinol LVII, the tertiary hydroxyl group of which is readily dehydrated to the diphenylethylene LVIII Chromic acid oxidation of the latter and saponification of the protecting acetyl radicles give nordesoxycholic acid (LIX) Application of the same reactions to the methyl ester of LIX leads to bisnordesoxycholic acid (LX) The procedures are again repeated with the methyl ester of the bisnor acid as far as the corresponding ternordiphenylethylene LXI, which, on ozonolysis, yields pregnane 3(α),12(α)-diol-20-one diacetate (LXII) Condensation of the activated 21 methyl group of LXII with benzaldehyde gives the benzal derivative LXIII, the ethylenic linkage of which undergoes oxidative cleavage with ozone to the keto aldehyde LXIV (not isolated) Periodic acid oxidation of the latter then furnishes the diacetate (LXV) of etiodesoxycholic acid (LIV)

Several more promising approaches to the degradation of the bile acid side chain have recently been described by Jacobsen (113), Miescher (169,170),[4] Gallagher (106), and Wallis (12), and collaborators. Through the application of the salient features of each, it is apparent that the desired immediate starting materials (LIII, LIV, and LV) for the preparation of the cortical hormones may be realized in more satisfactory yield and in fewer operations than by the uneconomical and cumbersome method outlined in Table X

2 *Introduction of Oxygen at C 11*

The desired ketonic or alcoholic function may be introduced at C-11 through the mediation of the $\Delta^{9\,11}$ or $\Delta^{11\,12}$ double bond, or by bromination of the reactive methylene group at C-11 in the 12 keto bile acid series

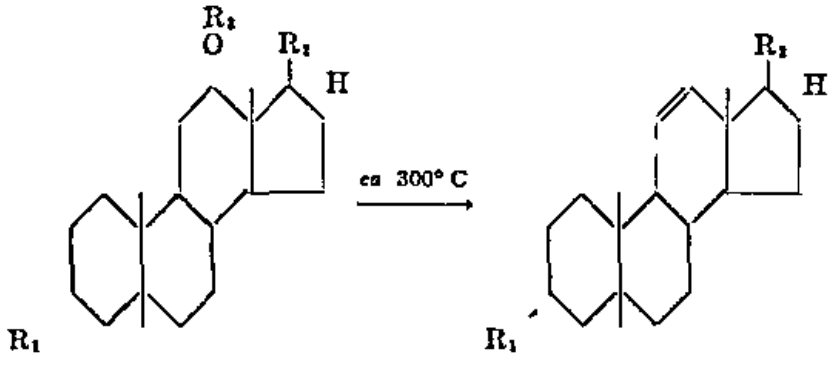
Reichstein and collaborators (1,14,93,94,123,125,128,129) and McKenzie, McGuckin, and Kendall (175) effected (Table XI) pyrolytic removal of the 12 hydroxyl group of desoxycholic acid and other 12-hydroxylated steroids (LXVI) to the corresponding 11,12 unsaturated compounds (LXVII) While the fission proceeds with the free alcohol,

[4] Full details of the elegant method of Meystre *et al* (169,170) have only recently been received Treatment of the diphenylethylene LVIII (Table X) with *N* bromosuccinimide yields the corresponding 22-bromo derivative from which hydrogen bromide may be readily removed to the $\Delta^{20\,22}$-diene Oxidation of the latter with chromic acid then affords the desired methyl ketone LXII in fewer steps than by the Barbier Wieland degradation and in vastly improved yield

better yields are obtained from the 12 benzoate or the 12-anthraquinone-β carboxylate. The reaction is general, and has been applied with a hydroxyl, acetoxyl, or carbonyl function at C-3 and to compounds bearing at C-17 the methyl ketone side chain or that of the bile acids, nor acids, bisnor acids or etio acids (mainly as esters)

TABLE XI

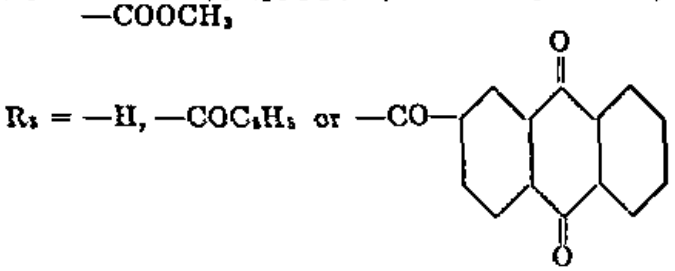
PYROLYSIS OF 12-HYDROXY STEROIDS



LXVI LXVII

R_1 = =O, —OH or —$OCOCH_3$

R_2 = —$CHCH_2CH_2COOCH_3$, —$CHCH_2COOCH_3$, —$CHCOOCH_3$, —$COCH_3$ or —$COOCH_3$



Treatment (Reichstein and collaborators, 94,124,126,128,129,179,-196,199) of the Δ^{11}-ethylenic compound LXVII with *N*-bromoacetamide or with hypobromous acid leads to a mixture, usually not separated, of the 11,12 dibromide LXVIII, the 9,11-dibromo-12 hydroxy derivative LXIX, and the desired bromohydrin LXX After oxidation of the hydroxyl groups to the corresponding ketones with chromic anhydride, the mixture is debrominated with zinc dust to give unchanged starting material (LXVII), the corresponding $\Delta^{9,11}$-12-keto derivative LXXI, and the desired 11-keto compound LXXII, separated by chromatographic fractionation As in the case of the pyrolysis, the bromohydrin reaction is applicable to 3-keto and 3 acetoxy compounds with the etio-, bisnor , nor or bile acid side chain, or with the methyl ketone side chain

(LXVII, Table XI) After protection of the 3-hydroxyl group of LII (or its methyl ester) by limited acetylation (116,224) to the 3-monoacetate (LXXIII), or by the action of succinic anhydride, which fails to esterify the 12-hydroxyl group (223), the 12-keto derivative LXXIV is obtained on oxidation with chromic anhydride Bromination of LXXIV and dehydrobromination on refluxing with pyridine, collidine, or sodium ethylate give 3(α)-hydroxy (or acetoxy)-12 keto $\Delta^{9\,11}$-cholenic acid (LXXV or LXXVI) (29,95,135 224) Alternately, the double bond may be introduced directly by dehydrogenation with selenium dioxide This elegant method of Schwenk has been applied by Kendall (116) to the methyl ester of LXXIV to afford the methyl ester of LXXVI in 90% yield Reduction of LXXVI with sodium ethylate by the Wolff-Kishner method provides $\Delta^{9\,11}$-lithocholenic acid (LXXVII) Like the 11,12-ethylenic linkage, the 9,11 double bond may be oxygenated at C-11 through the addition of the elements of hypobromous acid, and application (Hicks and Wallis, 96) of the Reichstein procedure (Table XII) to the acetate methyl ester of LXXVII leads to the same 3(α)-acetoxy-11-ketocholanic acid methyl ester

TABLE XII

INTRODUCTION OF 11 OXYGEN ATOM (METHOD OF REICHSTEIN)

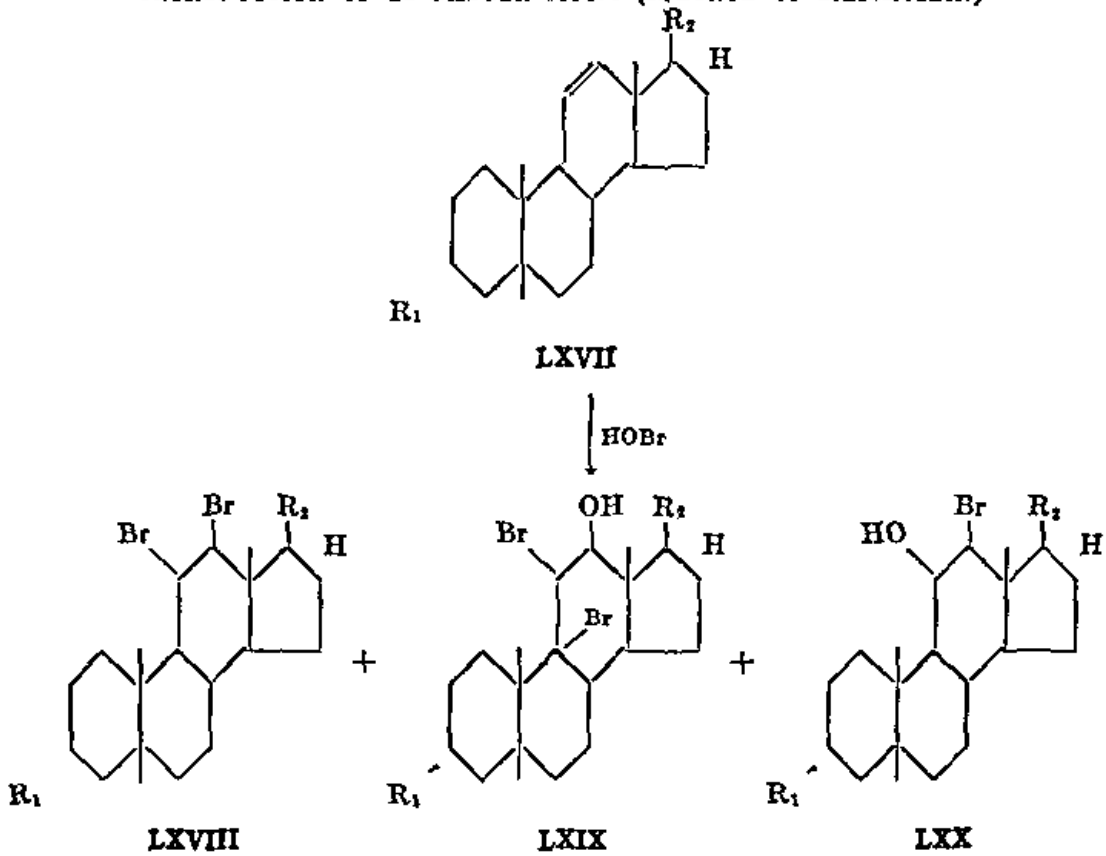
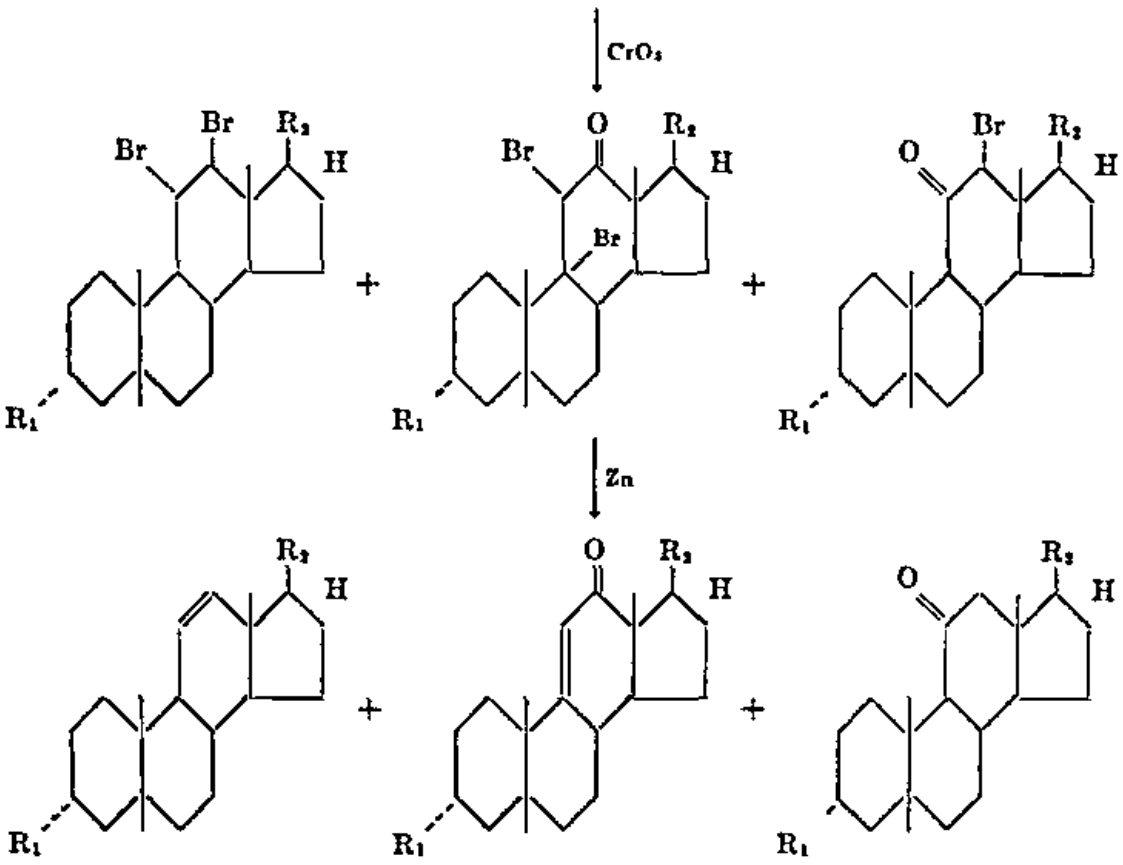


TABLE XII (*Continued*)



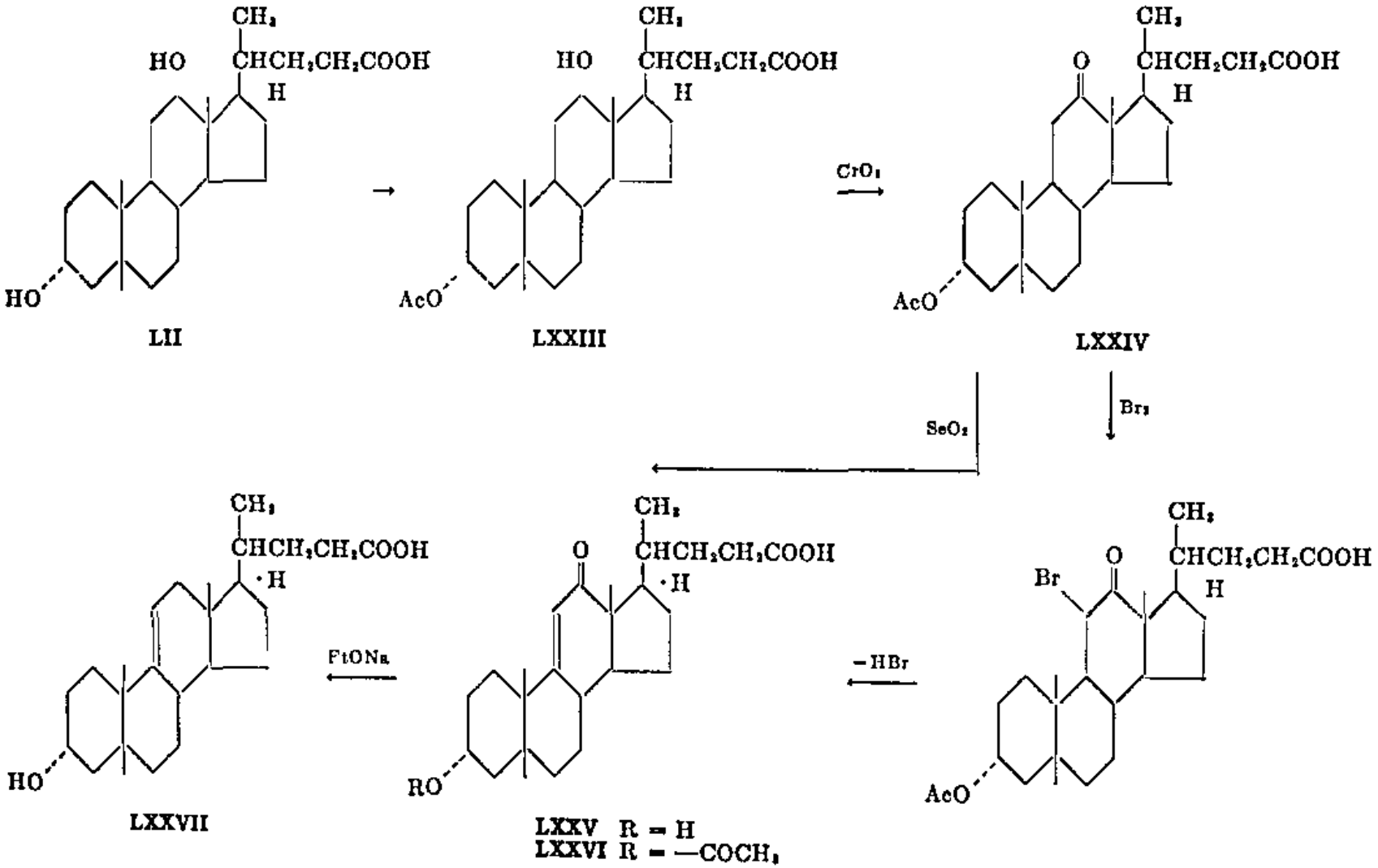
LXVII LXXI LXXII

R_1, R_2 as in Table XI

Kendall and associates (116) have elaborated (Table XIV) a unique method of 11-oxygenation which in addition affords worthwhile advantages in the degradation of the side chain Catalytic hydrogenation in ethanol–acetic acid (1 1) of the methyl ester (LXXVIII) of 3(α)-hydroxy-12-keto-$\Delta^{9,11}$-cholenic acid (LXXV), prepared as indicated in Table XIII, quantitatively reduces the 12-ketone group to the alcohol without the saturation of the double bond The 12 hydroxyl group of LXXIX forms part of an allylic system and readily yields with methanol the 12-methyl ether LXXX, the methoxyl group of which is easily displaced with chlorine The highly labile methyl 3(α)-hydroxy-12-chloro-$\Delta^{9,11}$-cholenate (LXXXI) undergoes an extraordinary rearrangement in pyridine or in a two-phase mixture of water and chloroform Hydrogen chloride is eliminated and the ethylenic bond shifts to the 11,12 position with the formation of the 3,9-cyclic ether LXXXII Reference to scale models indicates that 3,9-epoxide structures of this type may arise only in the 3(α)-hydroxy bile acid series Bromination of the double bond of LXXXII at −50°C affords the dibromide LXXXIII, the 11-bromine atom of which is replaced by oxygen on treatment with silver chromate

TABLE XIII

Introduction of the 9,11 Ethylenic Linkage



Proof of the structure of LXXXIV is afforded by the opening of the epoxide ring to methyl 3(α)-acetoxy-11-keto-12-bromocholanate, identical with that obtained by the method of Reichstein (Table XII) The Barbier-Wieland degradation of the side chain (see Table X, page 576) proceeds more satisfactorily and in fewer operations in the epoxide series, as the 3-hydroxyl group is protected by the stable cyclic structure, which obviates the necessity of acetylation prior to the oxidations, and of saponification for each Grignard reaction The C-12 bromine atom in LXXXIV is eliminated in the course of the first Grignard reaction By this route, 3,9-epoxy-pregnane-11,20-dione (LXXXV) and 3,9-epoxy-11-ketoetiocholanic acid (LXXXVI) may be obtained in excellent yield However, opening the epoxide ring becomes more difficult as the side

TABLE XIV

INTRODUCTION OF THE 11-OXYGEN ATOM (METHOD OF KENDALL)

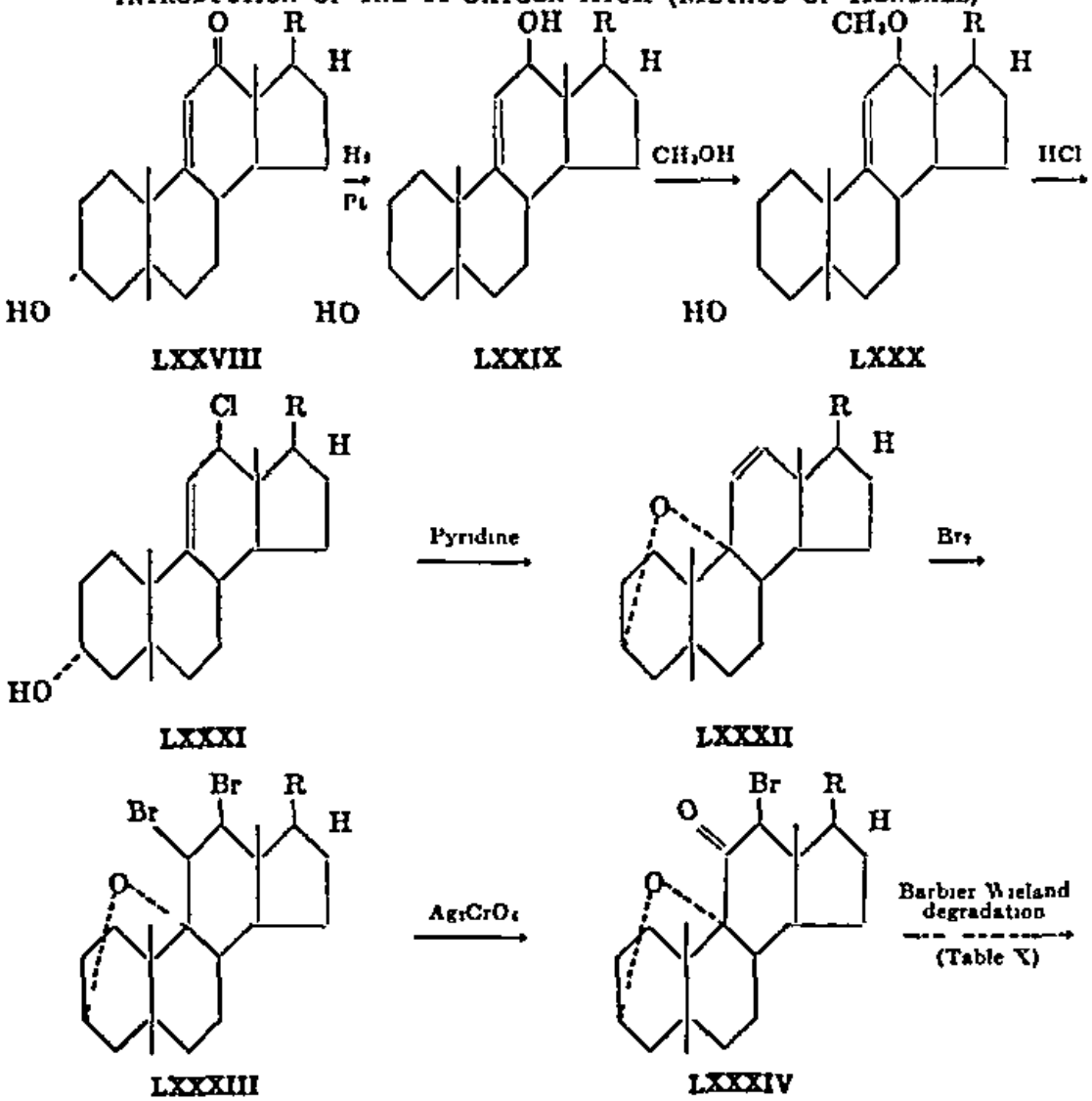
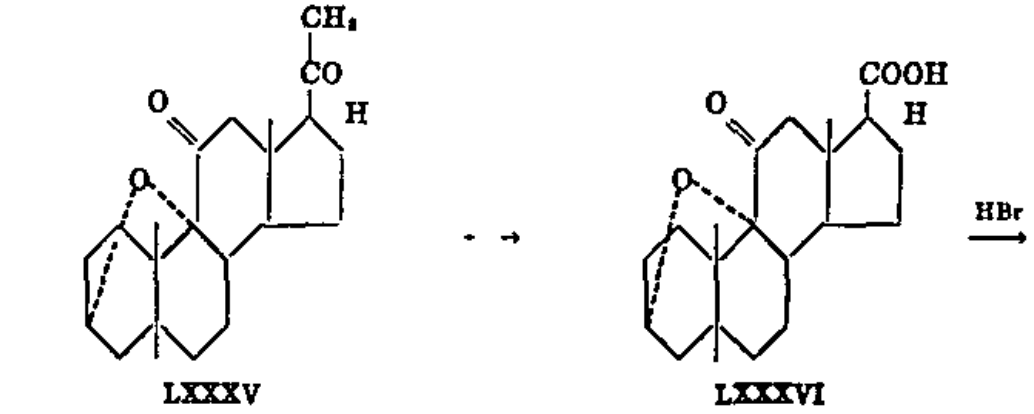
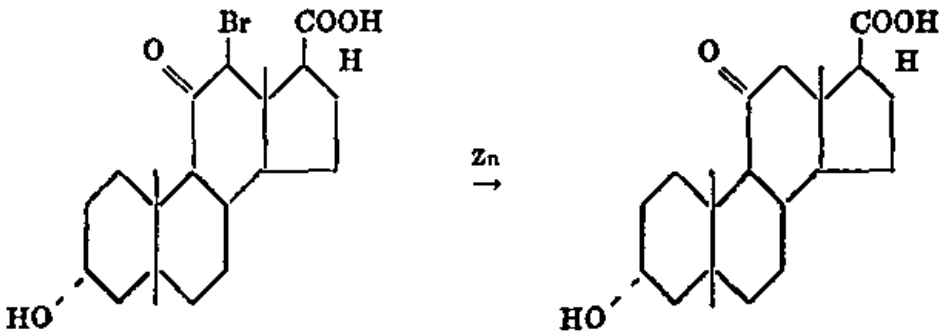


TABLE XIV (*Continued*)



LXXXV LXXXVI



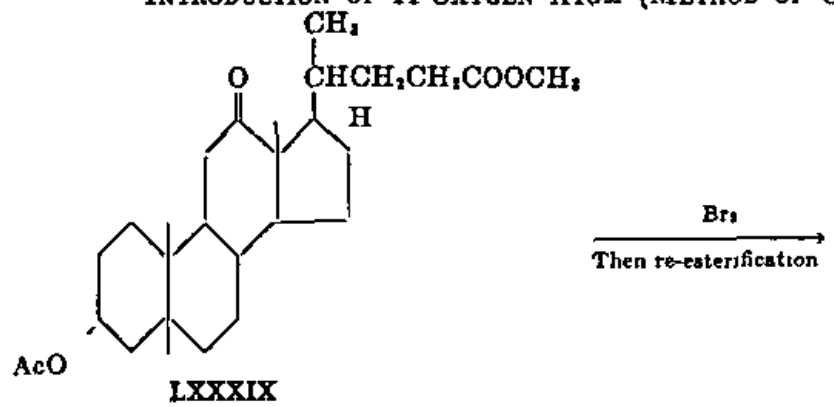
LXXXVII LXXXVIII

$$R = -\underset{\displaystyle CH_3}{\overset{|}{C}}HCH_2CH_2COOCH_3$$

chain is shortened This is accomplished by the addition of hydrogen bromide to the intermediate LXXXVI which is debrominated with zinc to 3(α)-hydroxy-11-ketoetiocholanic acid (LXXXVIII) As the reaction proceeds in poor yield at the etio acid stage, it is more profitable to open the epoxide ring of the nor acid and continue the degradation from there, as indicated in Table X.

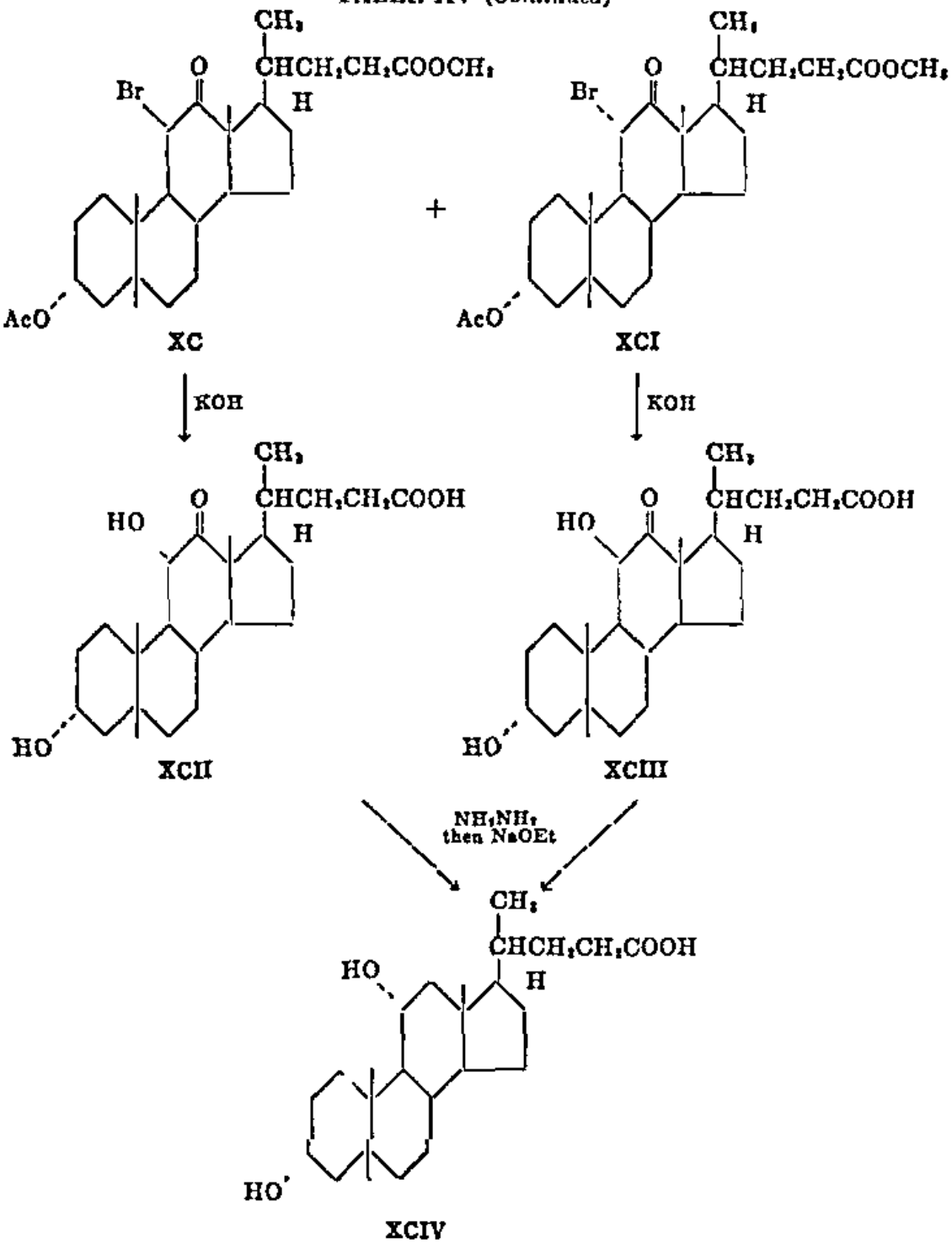
TABLE XV

INTRODUCTION OF 11 OXYGEN ATOM (METHOD OF GALLAGHER)



LXXXIX

TABLE XV (*Continued*)



Gallagher and co-workers (69,71) have developed an efficient method (Table XV) of 11-oxygenation by direct bromination of the methyl ester (LXXXIX) of 3(α)-acetoxy-12-ketocholanic acid (LXXIV), prepared as outlined in Table XIII On treatment with bromine in glacial acetic acid at room temperature and re-esterification, the 11(α)-bromo deriva-

tive XCI is formed predominately, with smaller quantities of the 11(β) epimer, XC Saponification of each at room temperature leads, respectively, to 3(α),11(β)-dihydroxy-12-ketocholanic acid (XCIII) and 3(α),11(α)-dihydroxy-12-ketocholanic acid (XCII), Walden inversions occurring in the course of the hydrolyses Wolff-Kishner reduction of the hydrazones of both XCII and XCIII gives the desired 3(α),11(α)-dihydroxycholanic acid (XCIV) together with smaller amounts of Δ^{11}-lithocholenic acid, lithocholic acid, and 3,11,12-trihydroxycholanic acids Presumably, in the alkaline reaction medium, the 11(α)-hydroxy compound represents the stable component of an epimeric equilibrium mixture, while the labile 11(β)-hydroxy isomer suffers dehydration

3 *Partial Synthesis of 11-Dehydrocorticosterone*

Methyl 3,11-diketoetiocholanate (XCV), obtained from etiodesoxycholic acid methyl ester as illustrated in Table XII, hydrogenates (126) predominately to the 3(β) epimer XCVI From the acetate of the corresponding free acid (XCVII), Lardon and Reichstein (127) built up the α-ketol side chain by the route previously employed in the preparation of desoxycorticosterone (Table VIII, page 571) After oxidation of the 3-hydroxyl group of pregnane-3,21-diol-11,20-dione 21-acetate (XCVIII) to the corresponding 3-ketone XCIX, the Δ^4-ethylenic

TABLE XVI

PARTIAL SYNTHESIS OF 11 DEHYDROCORTICOSTERONE

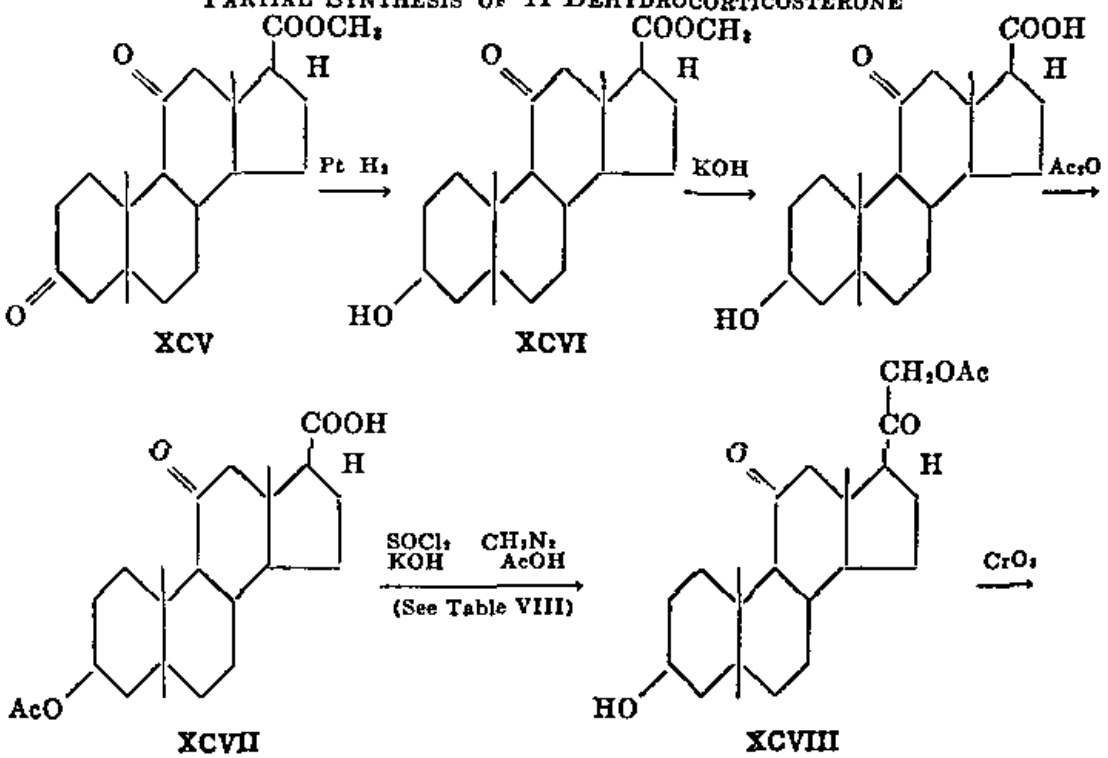
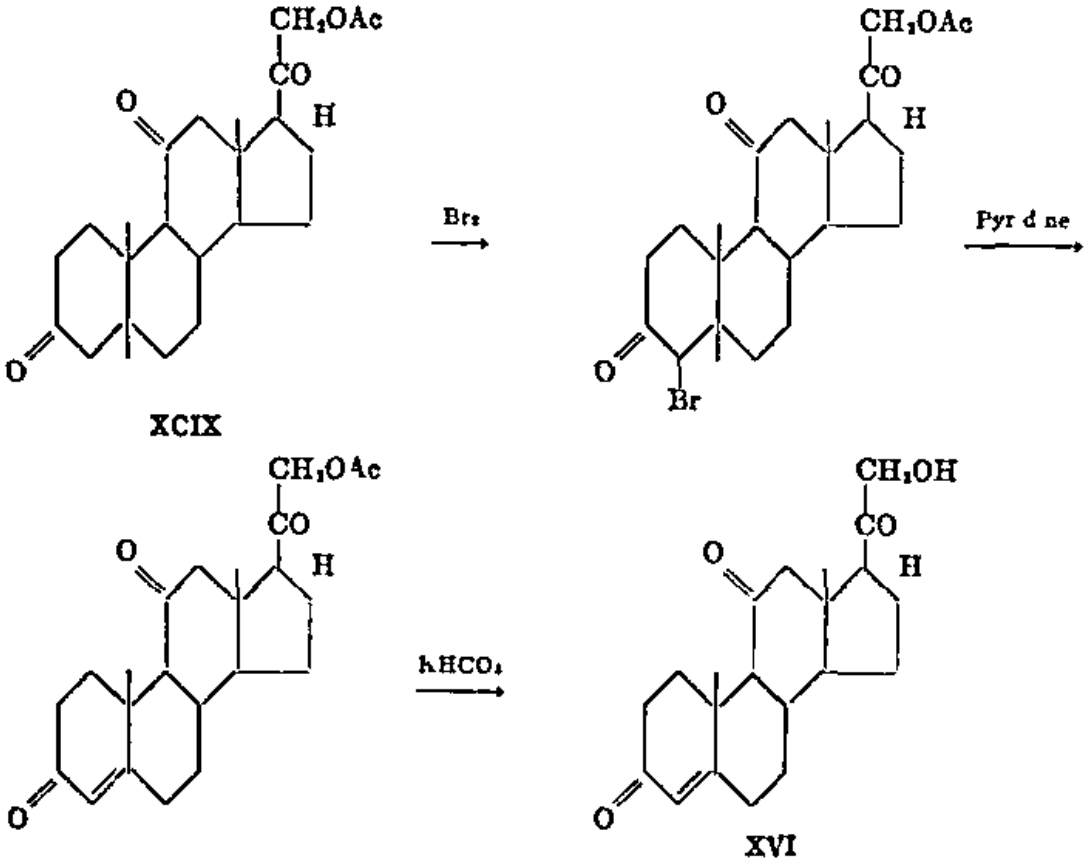


TABLE XVI (*Continued*)



linkage was introduced by bromination at C-4 and dehydrobromination to XVI, identical in all respects with natural 11-dehydrocorticosterone

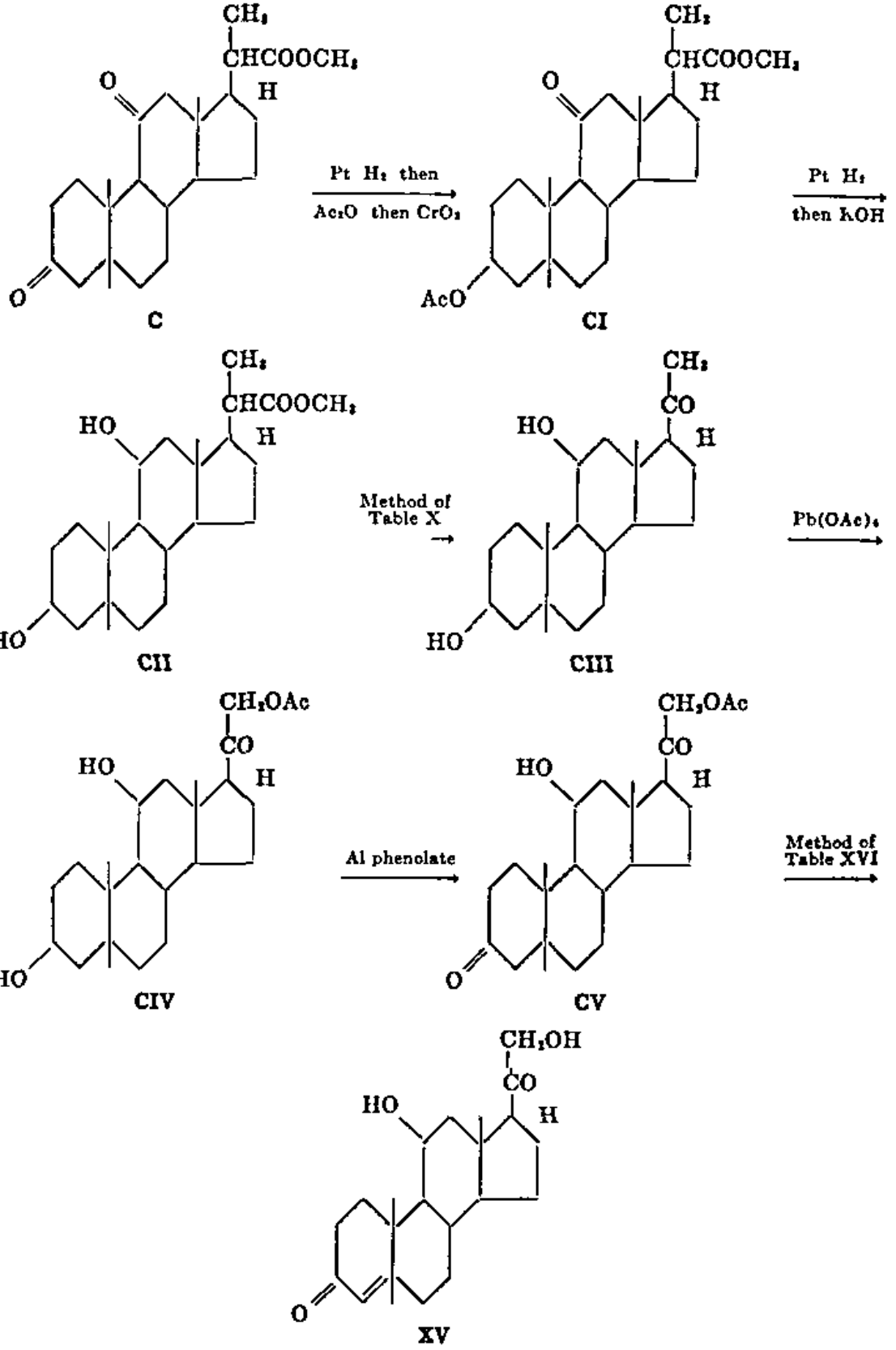
Gallagher (68) and Kendall (private communication) have also prepared XVI by the degradation of the side chain of the 11 substituted bile acids obtained by their respective methods (Tables XV and XIV) and through the application of the reactions outlined in Table XVI

4 *Partial Synthesis of Corticosterone*

The synthesis of the α ketol side chain by the method outlined in Table VIII, page 571, is not applicable to the preparation of corticosterone (XV), as the non reactive 11 hydroxyl group cannot be protected by acetylation against the action of thionyl chloride or of oxidizing agents To circumvent the difficulty, von Euw, Lardon, and Reichstein (50) employed (Table XVII) the lead tetraacetate reaction of Dimroth and Schweizer (36), whereby a methyl group, activitated by a vicinal carbonyl, is oxidized to the corresponding acetoxyl derivative Methyl 3,11 diketobisnorcholanate (C), prepared from bisnordesoxycholic acid (LX) by pyrolysis (Table XI, p 579) and through the bromohydrin reaction (Table XII, pp 580–581), is hydrogenated (128) to 3(β),11(β) dihydroxybisnorcholanic acid methyl ester (CII), the reduction is carried

TABLE XVII

PARTIAL SYNTHESIS OF CORTICOSTERONE



out in two stages, the 3-ketone being hydrogenated under mild conditions to the 3(β) epimer CI predominately, and the 11-ketone under more energetic conditions principally to the non-reactive 11(β)-hydroxy epimer. Degradation (49) of the bisnor acid side chain by the method of Hoehn and Mason (Table X, page 576) then affords the corresponding methyl ketone CIII, which, on treatment with lead tetraacetate (36), gives the α-ketol CIV in 25% yield The 3-hydroxyl group of CIV is preferentially oxidized with aluminum phenolate to CV, which permits the introduction of the Δ^4-ethylenic linkage in the usual way by bromination and dehydrobromination (see Table VIII, page 571) The final product (XV) is identical with natural corticosterone

5 *Partial Synthesis of 17-Hydroxy-11-Dehydrocorticosterone*

Kendall's Compound E (VIII, page 557) has recently been prepared (as acetate) by Sarrett (219) (Table XVIII) Desoxycholic acid (LII) was degraded to the bisnor acid (LX) by the procedure of Hoehn and Mason (Table X), at which stage the 11-ketone group was introduced by the Reichstein method (Tables XI and XII, pages 579 581) to

TABLE XVIII

PARTIAL SYNTHESIS OF 17 HYDROXY 11 DEHYDROCORTICOSTERONE

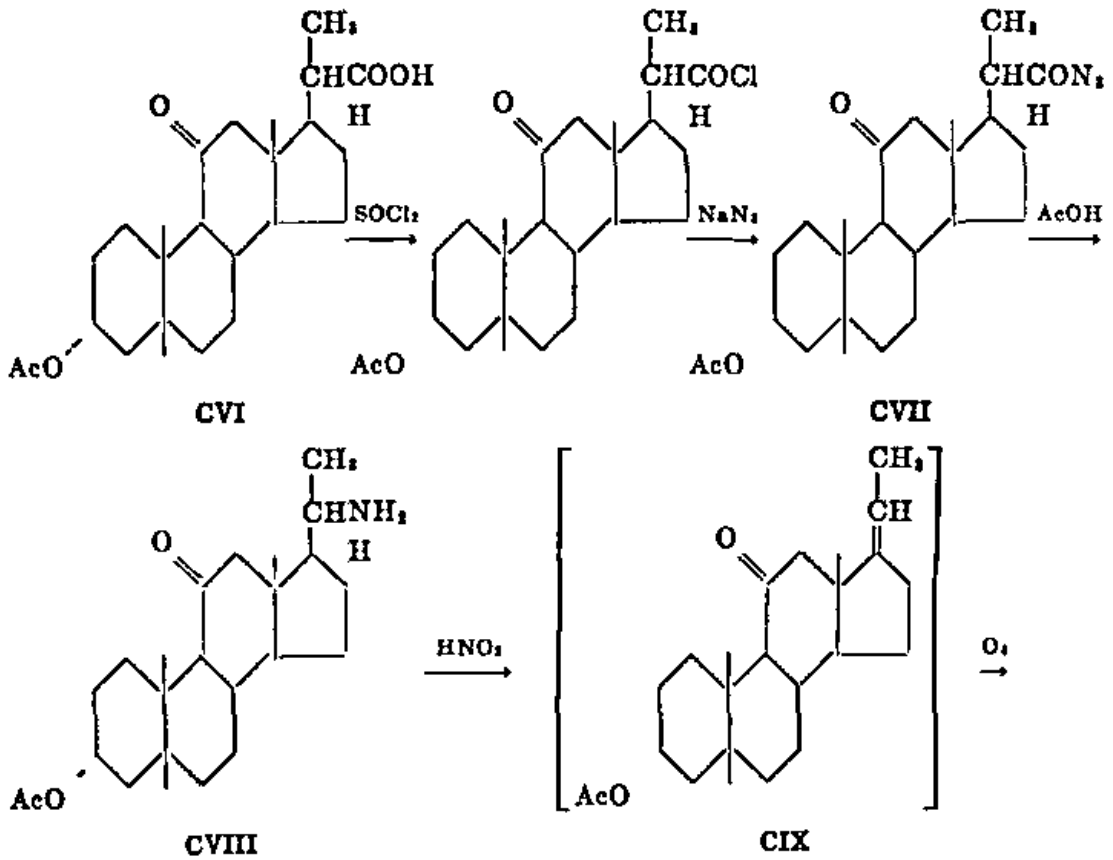


TABLE XVIII (*Continued*)

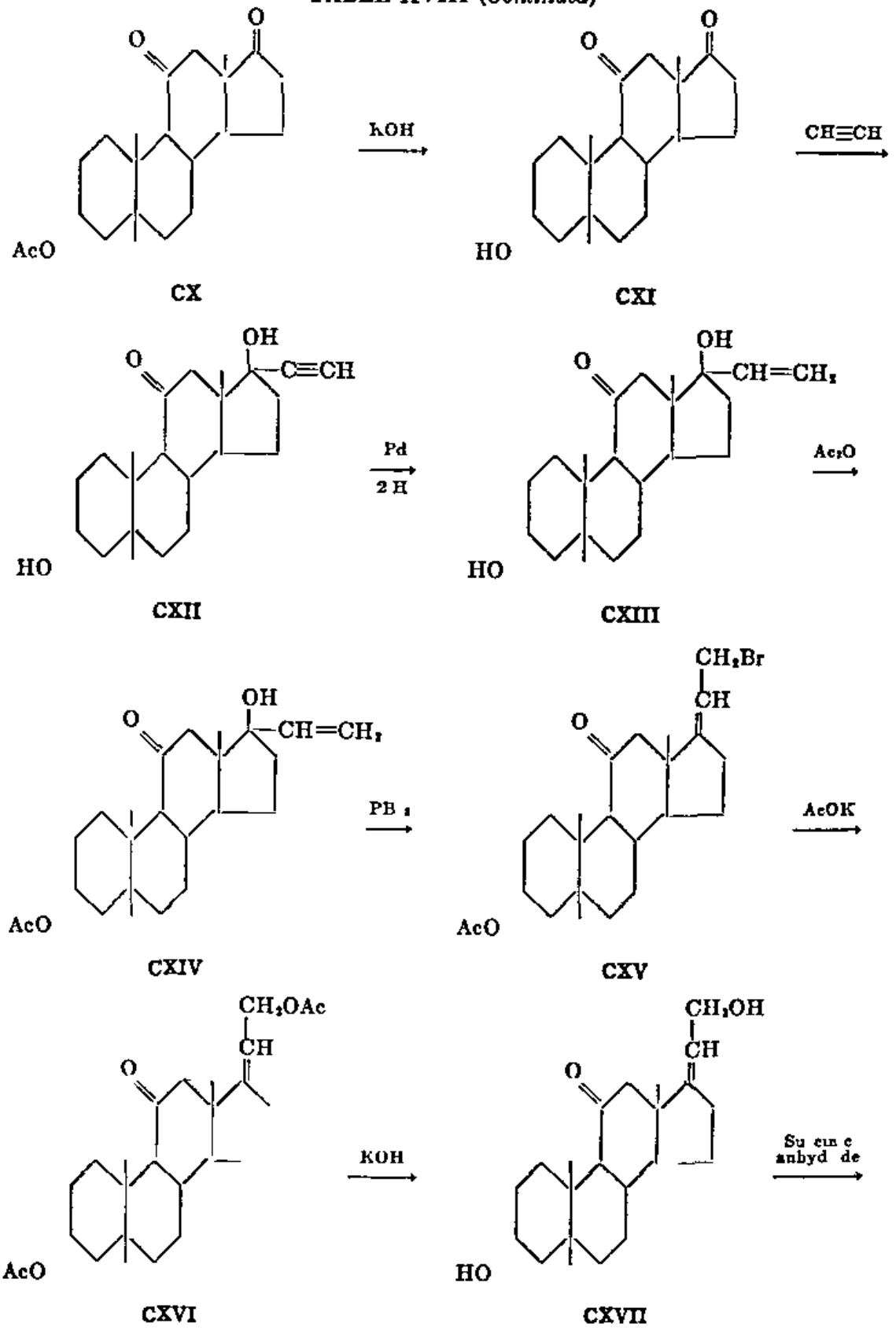
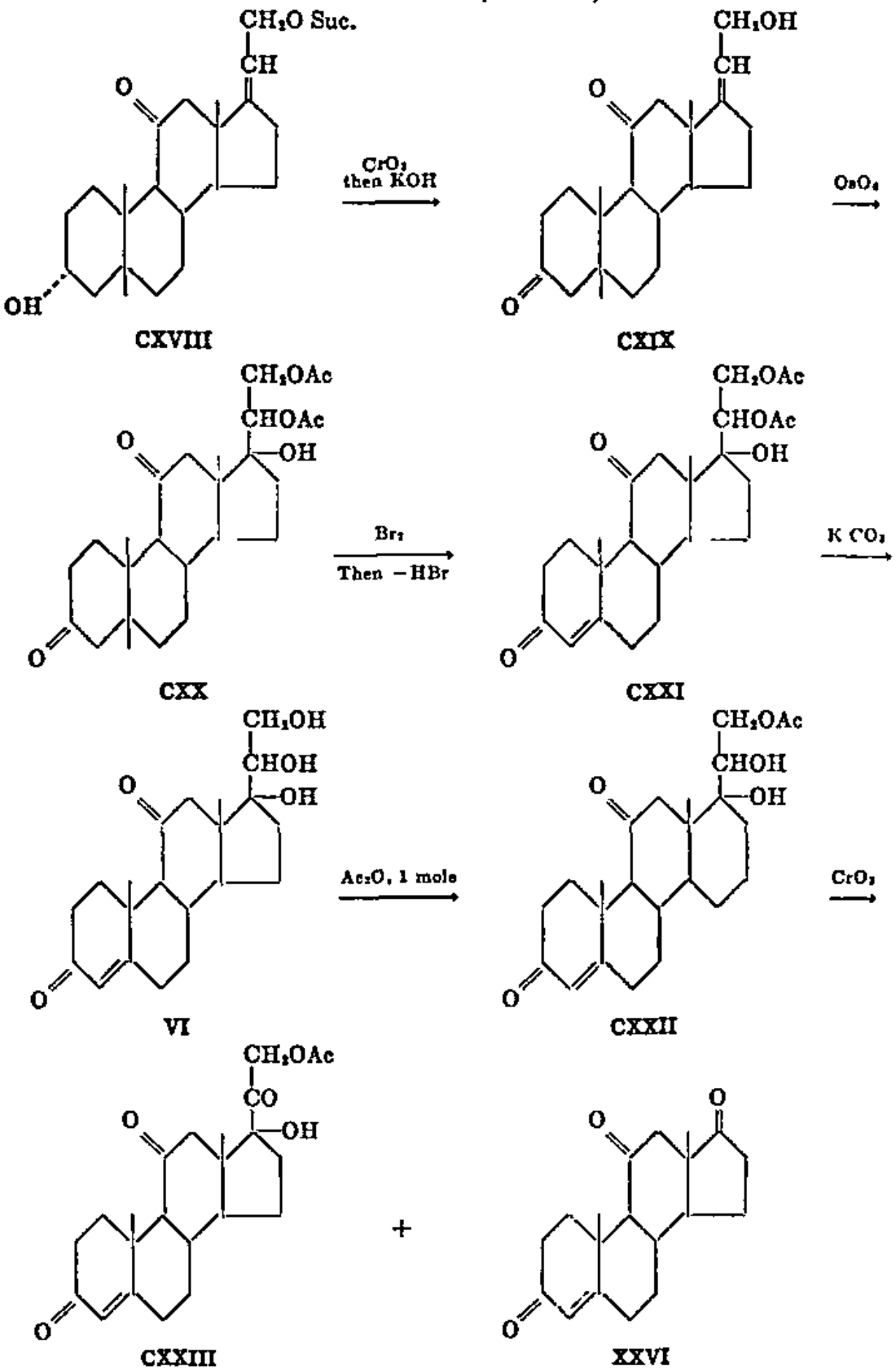


TABLE XVIII (*Continued*).



furnish the immediate starting material 3(α)-acetoxy-11-ketobisnor cholanic acid (CVI) By means of a Curtius rearrangement, CVI was converted to the aminopregnane derivative CVIII, through the acid azide CVII Diazotization of the amine CVIII in aqueous pyridine gave an inseparable mixture of Δ^{16}-, Δ^{17}-, and Δ^{20}-pregnene derivatives, including the desired product CIX Ozonolysis of the mixture led to a ketonic fraction from which etiocholan-3(α)-ol-11,17-dione acetate (CX) could be separated in crystalline state After saponification to the free alcohol CXI, acetylene added exclusively at C-17 to give the pregninediolone CXII, the acetylenic linkage of which was partially saturated with a palladium-barium carbonate catalyst to the pregnene derivative CXIII The procedure of Ruzicka and Muller (216) was then followed in the conversion of the 17 hydroxy Δ^{20}-pregnene, CXIV, to the 21-hydroxy-Δ^{17} derivative CXVII, through the intermediate 21 bromo and 21 acetoxy compounds, CXV and CXVI The 3-hydroxyl group is best oxidized to the ketone CXIX at this stage, protection being provided for the primary C_{21} alcohol by succinoylation (CXVIII) Hydroxylation of the double bond of the acetate of CXIX with osmium tetraoxide and acetylation of the product led to the trioldione diacetate CXX, in which the side chain at C-17 is oriented in the natural configuration The Δ^4-ethylenic bond was then introduced by bromination and dehydrobromination and the resulting α,β-unsaturated 3-ketone CXXI was saponified to the free trioldione VI, which is identical with the naturally occurring Compound U of Reichstein Chromic acid oxidation of the 21-monoacetate CXXII, obtained in good yield by limited acetylation of VI, then gave adrenosterone (XXVI) and CXXIII, identical with the 21-acetate of Kendall s Compound E (VIII)

The over-all yields from desoxycholic acid of dehydrocorticosterone, corticosterone, and Compound E are extremely minute (of the order 0 01 to 0 1% through the published partial syntheses outlined above) It is reasonable to anticipate however that the yields may be substantially increased by the incorporation into a single process of the best features of each different method and through improvements at all stages of the entire procedure

V. The Amorphous Fraction

After crystallization from gland concentrates (Sects II and III) of the active adrenal steroids mentioned above, there remain in the mother liquors substances which have resisted to date attempts at crystallization but which possess high activity per unit weight and which account for a significant proportion of the total activity of the original extract This non-crystalline syrup has been designated "the amorphous fraction"

(115,269), and obviously its composition will vary with different methods of fractionation, with species, and with the relative efficiencies of crystallization techniques By all methods, however, the factors retained in the water-soluble amorphous residue are, like desoxycorticosterone, highly potent with respect to life maintenance in the adrenalectomized test object and, unlike the 11-oxygenated adrenal hormones, are relatively inert with respect to carbohydrate metabolism (Table XX, page 595) The amorphous fractions described in 1935–1936 by Pfiffner, Wintersteiner, and Vars (185) and Wintersteiner and Pfiffner (269) maintained the adrenalectomized dog at a daily dose level of 0 0025 mg per kg , whereas 0 1 mg of 17-hydroxy-11-dehydrocorticosterone (VIII) failed to suffice In the experience of Mason (160) and of Kendall (114) the amorphous fraction retains about 50% of the activity of the original tissue extract, based on life maintenance in the adrenalectomized dog, and per unit weight is 7–15 times more active than desoxycorticosterone and 200–500 times more active than Compound E (VIII) in the same respect In the Everse-de Fremery test (54), the amorphous concentrates of Reichstein (205,208) exhibit about one fourth the activity of desoxycorticosterone and 3–4 times the activity of corticosterone Kuizenga (120) has synopsized (Table XIX) the distribution of activity in the course of the fractionation of extracts from two species with respect to (*a*) the work performance test of Ingle, which is essentially a measure of the 11-oxygenated active hormones, and (*b*) time-survival in the adrenalectomized rat, in which test desoxycorticosterone is the most potent of the

TABLE XIX

DISTRIBUTION OF ACTIVITY IN THE FRACTIONATION OF ADRENAL EXTRACTS[a]

Fraction	Hog extract			Sheep extract		
	Wt , g	Work test, units	Survival test, units	Wt , g	Work test, units	Survival test, units
Neutral ethyl acetate solution[b]	12 94	21,185	111,500	7 4	11,169	56,250
Benzene soluble portion	7 5	3,745 (18%)	24,000 (19%)	2 75	1,890 (17%)	7,312 (13%)
Resin and crystalline material insoluble in benzene	3 9	15,000 (66%)	58,500 (52%)	3 7	6,813 (61%)	27,000 (48%)
Water soluble amorphous fraction	0 8	800 (4%)	16,000 (14 3%)	0 65	450 (4%)	16,300 (29%)

[a] Data of Kuizenga (120)

[b] See Table I, page 552

known compounds The extraction procedure is that shown in Table I (page 552), followed by partition of the neutral fraction soluble in ethyl acetate between benzene and water The data clearly reveal the low proportion (4%) of carbohydrate-active substances in the water-soluble non-crystalline fraction and the relative accumulation therein of growth-survival material (14–29%)

The chemical nature of the active factors retained in the amorphous fraction through the usual extraction procedures is completely unknown, solubility properties and empirical analyses correspond with compounds of the $C_{21}O_5$ series

VI Relationship between Chemical Structure and Physiological Action

Three structural features appear to be essential to a high degree of activity with respect to life maintenance and salt and water metabolism, namely (*a*) the $\alpha\beta$-unsaturated 3-ketone grouping, (*b*) a reducing α ketol grouping in the side chain, and, (*c*) the stable orientation of the side chain at C-17, to these must be added a fourth requisite to appreciable glycogenic potency, *i e*, (*d*) an alcoholic or ketonic oxygen atom substituted at C 11

A number of non steroid α ketols have been prepared by Linnell and Roushdi (132) but of these only the compound CXXIV (Table XX), a close structural relative of stilbestrol, exhibited slight activity in the life maintenance test in young adrenalectomized rats (about $\frac{1}{200}$ of the potency of desoxycorticosterone acetate) In Table XXI is set out a comparison, by various methods of assay, of the potencies of the six most active adrenal steroids and the amorphous fraction, while Table XX lists the formulas of a number of compounds, closely related chemically to the active adrenal steroids the bioassays of which have contributed greatly to the present understanding of the relationship between constitution and physiological action

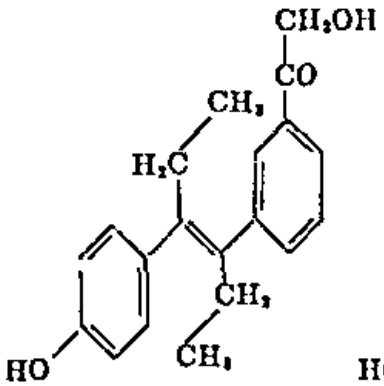
A Rings A and B

As with the progestational and androgenic hormones (Chapters XI and XII, respectively) most marked potency is associated with those compounds which possess the 4 5-ethylenic linkage in conjugation with a carbonyl oxygen atom at C-3 In all six of the highly active adrenal steroids (Table XXI) this resonance system obtains Saturation of the 3-ketone or of both the 3-carbonyl group and the 4 5-ethylenic bond leads to a significant diminution of activity Of the ring A-saturated adrenal steroids which retain the α ketol side chain (compounds II, III, IV, X, XII, and XIII), only compounds III and IV have been examined

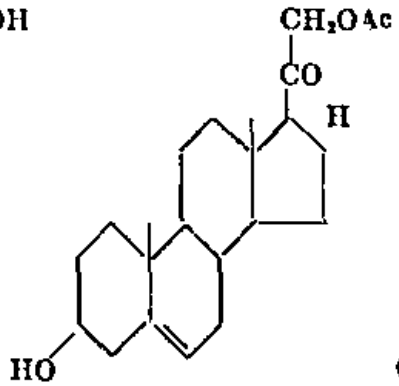
biologically In the Everse-de Fremery test both proved to be inactive in relatively small doses (201) Likewise, and in small amounts, IV failed to prolong survival in adrenalectomized rats, but III showed considerable activity in this regard (121), an observation which merits further examination Mason *et al* (162) hydrogenated dehydrocorticosterone (XVI) and corticosterone (XV) with palladium black conditions which may be expected to lead to the reduction of the double bond only,

TABLE XX

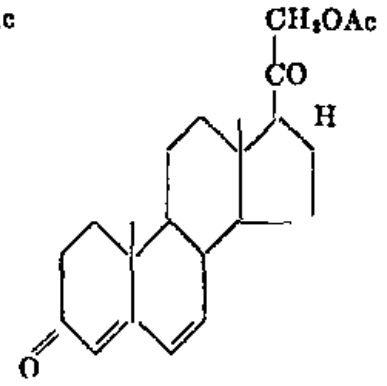
COMPOUNDS RELATED TO THE ADRENAL STEROIDS



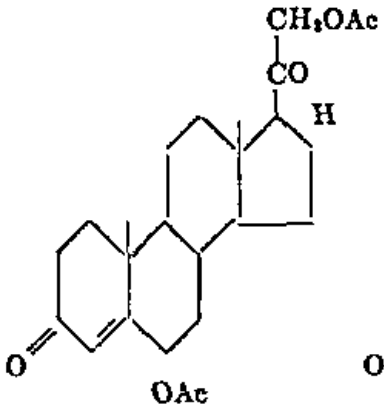
CXXIV



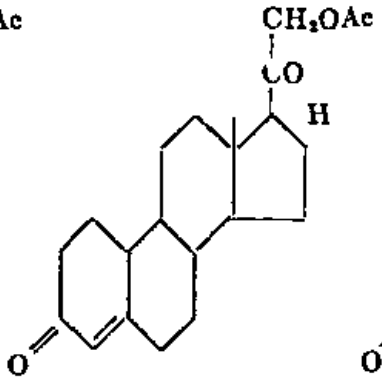
CXXV



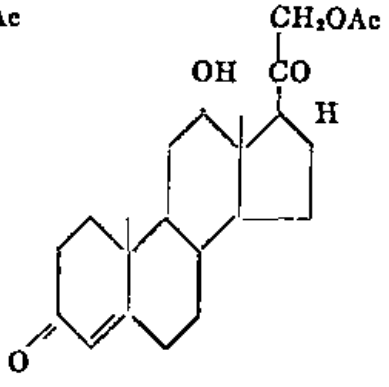
CXXVI



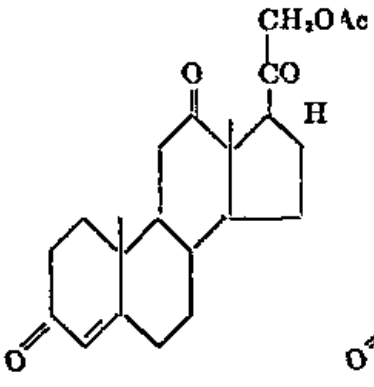
CXXVII



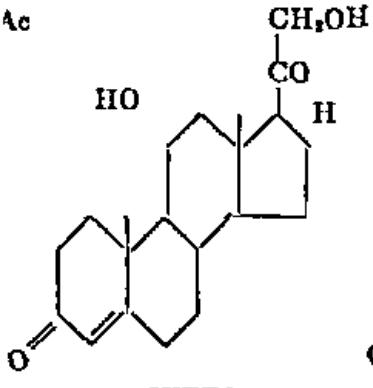
CXXVIII



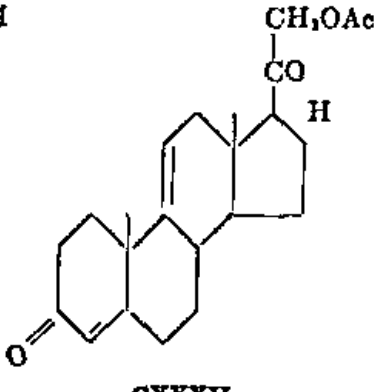
CXXIX



CXXX

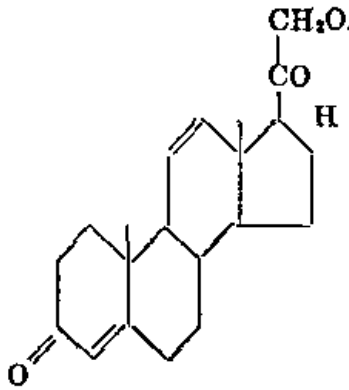


CXXXI

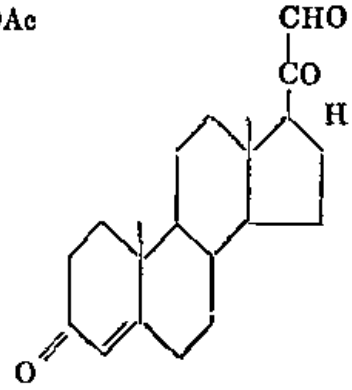


CXXXII

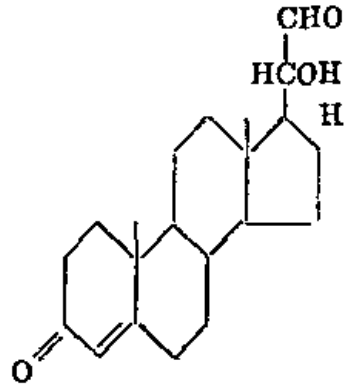
TABLE XX (*Continued*)



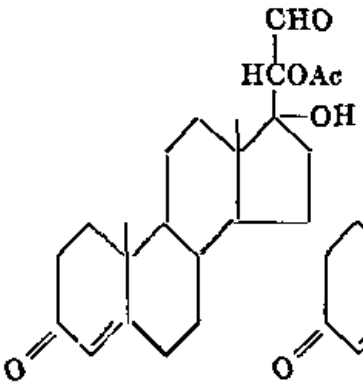
CXXXIII



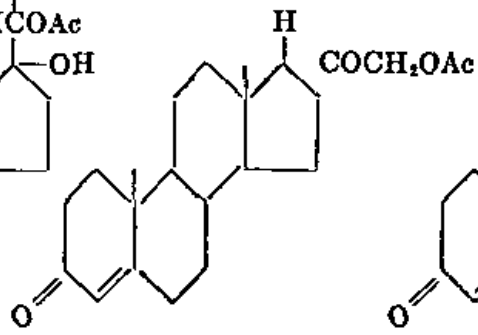
CXXXIV



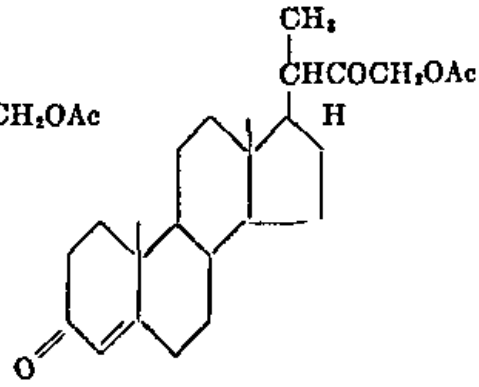
CXXXV



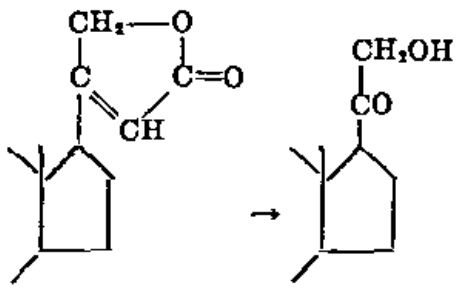
CXXXVI



CXXXVII



CXXXVIII



CXXXIX CXL

and observed thereby a fiftyfold diminution in activity in each instance (Ingle test) Similarly, reduction of the ethylenic linkage of desoxycorticosterone acetate leads to a mixture of 21-acetoxypregnane-3 20-dione and 21 acetoxyallopregnane-3,20-dione, both of which are inactive (life maintenance 266) 21-Acetoxypregnenolone (CXXV) was observed by Waterman *et al* (260) to possess some activity in adrenalectomized dogs, the potency relative to desoxycorticosterone acetate being 1 6 (31), in the survival test in adrenalectomized rats (225,226) and guinea pigs (13) the relative activities are approximately 1 3 and 1 5 Compared to desoxycorticosterone acetate with respect to increased work performance in the adrenalectomized rat, CXXV is practically inert (109)

Certain of the cardiac glycosides (ouabain, digitalin, and strophanthin), which do not contain in the molecule the α,β unsaturated 3 ketone grouping have been reported by Zwemer, Loewenstein, and Pines (271) to possess cortical action in that, in intact animals, they protect against insulin shock and lethal doses of potassium, and cause a rise in blood sugar and fall in blood potassium and protein Indefinite maintenance of adrenalectomized animals was not clearly demonstrated, nor have these glycosides been shown to be active in the usual methods of assay in the adrenalectomized test object

Unsaturation and substitution in ring B adversely influence the resonance system with loss of physiological potency Thus the diene ketone, 6-dehydrodesoxycorticosterone acetate (CXXVI), prepared by Wettstein (264), is inactive (survival test in rats) in doses of 1 mg per day (under the same conditions 0 25 mg desoxycorticosterone acetate gives a full response) 6 Dehydroprogesterone, however, exhibits approximately 50% of the progestational activity of progesterone (264) Ehrenstein (46) has prepared 6(α)-acetoxydesoxycorticosterone acetate (CXXVII), the effective dose of which in life maintenance in adrenalectomized rats is 10–20 times that of desoxycorticosterone acetate, also the product is without diabetogenic action and without influence in the work performance test of Ingle at levels of 1 and 2 mg per day, respectively

Likewise the absence of the angular methyl group at C-10 diminishes or abolishes activity 10 Nordesoxycorticosterone acetate (CXXVIII) is without effect on life maintenance or work performance at a dosage level approximately three times the minimal effective dose of desoxycorticosterone acetate (47) This stands in contrast to 10 norprogesterone, the progestational potency of which is equal to or greater than that of progesterone (47)

B Ring *C*

It is apparent from the relative activities of the cortical steroids shown in Table XXI that only those compounds bearing at C-11 an alcoholic or ketonic group possess power to cause deposition of glycogen in the liver of the adrenalectomized animal Similarly, marked activity *in the Ingle work test and high anti insulin and diabetogenic action is* confined to the same group of compounds In the absence of the 11 oxygen atom, the effect on carbohydrate metabolism, as evidenced by the above mentioned tests, is markedly diminished, conversely the 11 desoxy group and the amorphous fraction exhibit the highest degree of activity with respect to life maintenance and in the Everse-de Fremery test As regards sodium chloride excretion in intact animals, an alcoholic or ketonic function at C-11 decreases sodium retention (251)

Transposition in ring C of the oxygen atom from C-11 to C-12 leads

TABLE XXI

COMPARISON OF THE ACTIVE CORTICOIDS BY DIFFERENT METHODS OF BIOASSAY[a]

Compound	Formula	Designation		Assay Method							
		Kendall	Reichstein	Everse-de Fremery test,[b] mg /unit	Survival test,[c] mg / unit	Na^+ and Cl^- retention[d]	Ingle test,[e] mg / unit	Anti-insulin test[f]	Diabetogenic action[g]	Glycogen deposition: Method of Olson *et al*,[h] mg	Glycogen deposition: Method of Venning *et al*,[i] mg
Corticosterone	XV	B	H	1 0	0 13	++	0 42	+++	+++	0 77	
17-Hydroxycorticosterone	VII	F	M	1 5 to 2 0	0 10	– – – –	0 16	++++		0 51	
11-Dehydrocorticosterone	XVI	A			0 17		0 60	+++	+++	0 67	0 050
17-Hydroxy-11-dehydrocorticosterone	VIII	E	Fa	2 0	0 10	– – – –	0 20	++++	++	0 57	0 016
11-Desoxycorticosterone	XXII		Q	0 07[j]	0 025	++++	10 0	– –	– –	Inactive at 1 20[j]	
17-Hydroxy-11-desoxycorticosterone	XI		S	1 0[j]	0 13		10 0				
Amorphous fraction	($C_{21}O_4$?)			*ca* 0 3	0 04	?	1 7		?		

[a] Where figures are quoted in the vertical columns, the information has been gained within a single investigational group, and the values are therefore comparable, otherwise, and in instances where only roughly quantitative results are recorded, the use of the familiar plus and minus notation has been adopted

The data in this Table which represents an extension of the data of Reichstein and Shoppee (212), were selected to illustrate the relationship between physiological action and chemical structure in the corticoid series Accordingly, those assay methods are quoted in which the largest number of chemical entities has been examined Many other procedures are described which may exhibit greater sensitivity or specificity Also it should be emphasized that absolute values are relative to the state of the test object, conditions of administration, environment, diet, etc

[b] Recovery of the fatigued muscle in adrenalectomized rats Data recorded by Reichstein and Shoppee (212)

[c] Adrenalectomized rats Data recorded by Kuizenga (120)

[d] Intact dog Data of Thorn, Engel, and Lewis (251)

[e] Work performance under the influence of repeated faradic stimuli in adrenalectomized rats Data recorded by Kuizenga (120)

[f] Prevention of insulin convulsions in intact rats Data of Grattan and Jensen (76)

[g] Increased glycosuria in partially depancreatized and in adrenalectomized depancreatized rats Data of Long, Katzin, and Fry (133)

[h] Deposition of liver glycogen in fasted, adrenalectomized rats Data of Olson, Thayer, and Kopp (177), expressed as the quantity of hormone required to deposit 1 % liver glycogen

[i] Deposition of glycogen in the liver of the glucose treated, fasting, adrenalectomized mouse Data of Venning, Kazmin, and Bell (258), expressed as the quantity of hormone required to cause the deposition of 50 mg glycogen/100 g mouse (control glycogen level = *ca* 5 mg /100 g mouse)

[j] As 21 monoacetate

not only to loss of potency with respect to carbohydrate metabolism but also to diminished activity in the Everse-de Fremery test 12 Hydroxydesoxycorticosterone acetate (CXXIX, as 12-acetate) is without anti-insulin action in mice at a dose level at least eight times that effective with corticosterone (XV) acetate (67) The 12-keto analog CXXX is ineffective in the work performance test of Ingle (87) and with respect to the deposition of hepatic glycogen in adrenalectomized mice (87) at dosages 15 and 6 times, respectively, the active level of 11-dehydrocorticosterone (XVI) In the Everse-de Fremery test, CXXIX, as 12-acetate, and CXXX exhibit slight activity at about three times the effective level of corticosterone and forty times that of desoxycorticosterone acetate (67)

Epimerization of the 11 hydroxyl group of corticosterone apparently causes loss of potency 11 *Epi*corticosterone (CXXXI), as diacetate, has been prepared in non crystalline state by Gallagher (68) and preliminary tests indicate a low order of activity as measured by glycogen deposition and weight maintenance in adrenalectomized rats

Unsaturation in ring C does not markedly alter the potency in the Everse-de Fremery test Thus, the 9,11- and 11,12-ethylenic derivatives (CXXXII and CXXXIII) of desoxycorticosterone acetate are, respectively, 2–3 times more active and 2–3 times less active than the latter, in the survival-growth test in young rats, CXXXII is equally as effective (234)

Similarly in the progesterone series, 9- and 11-dehydroprogesterone retain pronounced progestational activity (93,233) The behavior of CXXXII and CXXXIII with respect to the deposition of liver glycogen (and in other cortical hormone assays) has not yet been reported

C The Side Chain

The reducing α ketol grouping of the side chain is essential to a high activity in all tests except life maintenance The effectiveness of progesterone (XXIV) in prolonging the period of survival of adrenalectomized animals was observed early by Gaunt and Hays (72), and has since been confirmed by many investigators and in several species, but the compound fails to protect against water intoxication (73), is without diabetogenic action (133), and is inactive at high dose levels in the Everse-de Fremery (243,260), anti insulin (76), and Ingle (110) tests

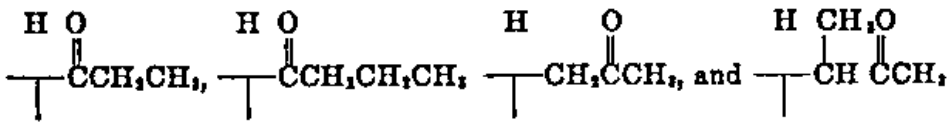
Replacement of the α-ketol with the reducing α-ketal grouping results in loss of potency The 21-aldehydo analog CXXXIV of desoxycorticosterone, prepared by Reich and Reichstein (198), is considerably less active than desoxycorticosterone (Everse-de Fremery test) Likewise, impure CXXXV, prepared by Mason (160) from corticosterone

(XV), and probably contaminated with the latter, is less active than corticosterone Similarly, reduction of the 20 carbonyl group to the secondary alcohol is accompanied by diminution in physiological potency, thus, Δ^4-pregnene-20,21-diol-3 one possesses less than one third the potency of desoxycorticosterone acetate (Everse-de Fremery test, 245) Also the hydroxy aldehyde CXXXV, prepared by von Euw and Reichstein (52), is markedly less active than desoxycorticosterone acetate in the Everse-de Fremery test (212)

Introduction of a tertiary hydroxyl group at C-17 (with the preservation of the normal orientation of the side chain) gives rise to marked diminution of potency in the life maintenance and Everse-de Fremery tests but enhances activity with respect to carbohydrate metabolism, this is evidenced by comparison of the appropriate pairs listed in Table XXI (*i e*, Compounds VII and XV, VIII and XVI, and XI and XXII) In the presence of an oxygen function at C-11, the ketol at 20–21, and the α,β-unsaturated 3-ketone groupings, the C-17 hydroxyl group induces sodium excretion (251)

The stable orientation at C-17 of the side chain is requisite to high activity Shoppee (230) has prepared 17 *iso*desoxycorticosterone acetate (CXXXVII), which is inactive (time survival test in rats) at a dosage three times the effective level of desoxycorticosterone acetate Similarly in the progesterone series, 17-*iso*progesterone is without luteoid action at a level at which progesterone is highly active (22)

Also substitution of the α ketol side chain directly at C-17 is apparently essential to physiological action The higher homolog CXXXVIII of desoxycorticosterone acetate, in which $-CH(CH_3)-$ is introduced between nucleus and the ketol grouping, is inactive (life maintenance test, 265) In the progesterone series, homologs have been prepared (171,172,192,265) with the side chains



all are without progestational activity with the exception of the first-mentioned, which exhibits about one third the potency of progesterone

The behavior of certain cardiac glycosides in preventing insulin shock has been referred to above These compounds do not possess the 2 carbon ketol side chain typical of the active adrenal steroids, but the conversion *in vivo* of the unsaturated lactone ring CXXXIX to the α-ketol system CXL by β oxidation has been cited by Reichstein and Shoppee (212) as a theoretical possibility

VII. Metabolism of the Adrenal Cortical Hormones

A Catabolism, Urinary Excretory Products as Indices of Adrenocortical Function

While relatively little information has been gained concerning the individual fate of the many steroids which have been isolated in crystalline state from the gland (Table III, page 557), cortical function has clearly been associated with the excretion of a large number of metabolites, many of which are of undetermined chemical structure These may be classified in two groups (*1*) those which may be quantitatively determined by the application of relatively simple colorimetric or bioassay techniques (and in this group fall an uncertain number of cortical metabolites of unknown structure, detectable only by virtue of a specific biological or chemical property), and (*2*) metabolites of established chemical constitution, estimable gravimetrically only after a usually tedious process of isolation The neutral 17-ketosteroids of urine occupy an intermediate position, in that the whole may be determined colorimetrically while the principal components of the mixture are known chemical substances which may be estimated individually by isolation In nearly all instances, the connection between urinary excretion and cortical function has been arrived at through the rise or fall in the output of a specific metabolite or group of metabolites (possessing a common property) under experimental or clinical conditions or hyper- or hypoadrenocortical function

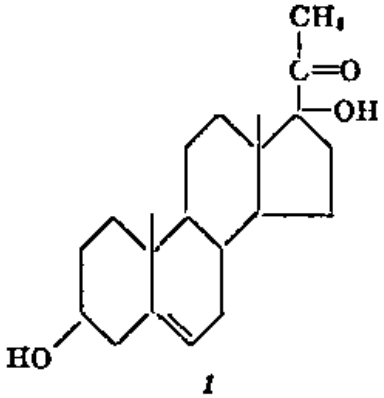
The following laboratory reactions, applicable to the water-insoluble, lipide-soluble, neutral products of urine, give a measure of the excretion of metabolites classified under group *1* but reflect cortical functions of quite different kinds (Table XXII, page 604) (*a*) 17-ketosteroids (Table XXIV, page 608), (*b*) cortin, (*c*) reducing substances, (*d*) 17-ketosteroids generated on periodic acid oxidation of the non-ketonic alcohols of urine, and (*e*) formaldehyde generated on periodic acid oxidation of the neutral fraction of urine And in group *2*[5], the following urinary excretory products (Table XXV, page 613) have definitely been associated (Table XXIII, page 606) with cortical function (*f*) Δ^5-androstene-3(β),17(α)-diol (CXLVIII), (*g*) Δ^5-androstene-3(β),16,17-triol (CXLIX), (*h*) Δ^5-pregnene-3(β) 20α diol (CL), (*i*) pregnane-3(α),20α-diol (CLI), (*j*) pregnane-3(α) 17,20 triol (CLII), and (*k*) pregnane-3(α),17-diol 20-one (CLIII)

[5] To the list of adrenal metabolites should be added Δ^4 pregnene-3(β),17 'β -diol 20-one (formula *1* below) isolated (5 3 mg /1) and characterized by Hirschmann and Hirschmann (100) from the urine of a boy with adrenal carcinoma The spatial arrangement at C-17 is that of the natural adrenal compounds (probably 17(α)OH, although formulated 17(β)OH by the authors) Accompanying the diolone was the

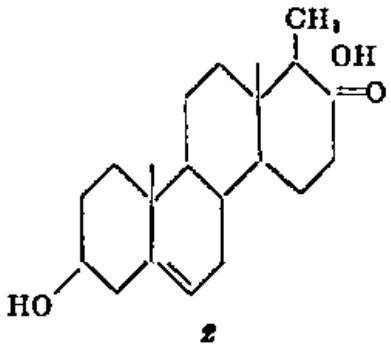
Of these, pregnane-3(α),20α-diol (CLI) is unique, in that this compound is also the chief metabolite in man of the corpus luteum hormone progesterone (XXIV), and that there exists a simple gravimetric method (253) for its estimation (as 3-monoglucuronide), which stands in contrast to the more elaborate chemical procedures requisite to the determination of the excretion of the other substances mentioned In addition to compounds *f* to *k*, several other very probable cortical metabolites have been separated from urine, but, to date, their relationship to cortical function may only be surmised, or they are characterized only by physical constants and elementary composition It is highly significant that of the ten or more recognized excretory products of the cortical hormones, only two, 11-hydroxy-androsterone (CXLVI) and 11-keto-etiocholan-3(α)-ol-17-one (CXLVII) (members of the 17-ketosteroid group *a*) have been shown conclusively to be oxygenated at C-11, but there can be no reasonable doubt that one or more of the components of "urinary cortin," which possesses carbohydrate activity, is also an 11-oxygenated compound which further fulfills the structural requirements at present associated with cortical activity of any type (Section VI)

Reactions *a* to *e*, which may be carried out routinely without chemical isolations, provide criteria of quite different kinds of cortical secretory function, which need not and do not run strictly parallel The neutral 17-ketosteroid colorimetric estimate gives a measure of the metabolites of the testicular and adrenocortical hormones excreted as 17-ketones, the quantity of cortical origin is about 10 mg per day Undoubtedly these products arise both from the catabolic breakdown of members of the C_{21} series and from the normal physiological secretion from the

corresponding *D*-homo rearrangement product (formula 2), 17a methyl Δ^4 *D*-homo-androstene 3(β),17a-diol 17-one (6 4 mg /1), which almost certainly arises from *1* in the course of the processing of the urine The close chemical relationship of *1* to the adrenal constituents 17"β"-hydroxyprogesterone (XXI) and allopregnane 3(α),-17 'β '-diol 20-one (XX, page 558), and the fact that *1* has not been encountered in normal urine leave little doubt that this excretory product arises from the adrenal cortex



1



2

TABLE XXII

DAILY URINARY EXCRETION OF CORTICAL METABOLITES

Subject	Criterion of cortical function					
	17-Ketosteroids,[a] mg	Cortin,[b] mg	Reducing substances, mg		Generated on HIO_4 oxidation	
			Heard *et al*[c]	Talbot *et al*[d]	17-Ketosteroids,[e] mg	Formaldehyde,[f] mg
Normal male average	15	0 062	1 5	0 24	0 4	0 5 to 0 8
Normal female average	10	0 039	1 3	0 24		
Hypoadrenalism						
Addison's disease	0–7	0 to 0 015		0 02 to 0 26		0 15
Panhypopituitarism	0–4	0	0 4 to 0 6	0 10 to 0 17		
Hyperadrenalism						
Hirsutism	15–30	0 050 to 0 065		0 23 to 0 32		
Cushing's syndrome	10–40	0 2 to 0 7	4 8	0 90 to 12 0		21 0
Virilism	40–250			0 15 to 0 57	10–16	
Stress						
Burns	20–30[g]	0 1 to 0 5[i]	3 0 to 4 0	0 34 to 1 70		
Postoperative	20–30[g]	0 1 to 0 2[i]		0 34 to 1 70		
Late pregnancy	10–20[h]	0 1 to 0 4	2 8 to 3 2			

[a] Expressed as the color equivalent of androsterone

[b] Data of Venning *et al* (92,254,251), expressed as the biological equivalent of Kendall's Compound E (VIII), compared by the power to cause deposition of glycogen in the liver of the adrenalectomized mouse

[c] Data of Heard, Sobel, and Venning (92), ascertained on extraction of the urine at pH 1 0 and expressed as the reducing equivalent of desoxycorticosterone

[d] Data of Talbot *et al* (250), ascertained by extraction of urine without acidification and expressed as mg of "corticosteroid"

[e] Data of Talbot and Eitingon (249), ascertained by extraction of urine with butanol and hydrolysis of the conjugates with barium chloride followed by hydrochloric acid or with liver enzyme, followed by barium chloride, followed by hydrochloric acid, expressed as the color equivalent of androsterone

[f] Data of Lowenstein, Corcoran, and Page (136) estimated by determination of formaldehyde generated on periodic acid oxidation of the neutral extract of acidified urine and calculated as the equivalent of dehydrocorticosterone (XVI)

[g] A slight rise, inconstantly observed immediately after damage, and followed in one or two days by a decline to normal or subnormal values (60,64,246)

[h] The upper limit is recorded by the Zimmermann reaction, and is probably due to increased output of 20-ketosteroids, no rise is observed by the antimony trichloride method (Venning, 254)

[i] Dependent on the severity of the stress, the rise, unlike that of the 17-ketosteroids, may persist for many weeks (137)

gland of members of the C_{19} series The active cortin like substance or substances are excreted in very much smaller amounts (of the order of 0 1 mg per day) and presumably represent an overflow of cortical hormones from the circulation Their chemical nature is not known but it is to be anticipated that the groupings essential to high cortical activity (Section VI) are contained in the molecules The urinary excretion of neutral water insoluble, chloroform soluble lipides which reduce cupric ion or phosphomolybdic acid also varies directly with degree of cortical function As reducing capacity is conferred by the primary α ketol grouping (side chain types *b* and *e* page 563), it is assumed that the determination provides a measure of the output of metabolites possessing

TABLE XXIII

URINARY EXCRETION OF RECOGNIZED ADRENOCORTICAL METABOLITES[a]

Metabolite	Formula	Normal urine	Adrenocortical lesion		
			Hyperplasia	Tumor	Carcinoma
Δ^5 Androstene-3(β) 17(α)-diol	CXLVIII			0 2 to 1 5	8–48
Δ^5 Androstene 3(β) 16 17 triol	CXLIX	0 1		20	20
Δ^5 Pregnene-3(β) 20α-diol	CL				35
Pregnane 3(α) 20α-diol	CLI	0 1	0 1–6	2 20	7–20
Pregnane 3(α) 17 20 triol	CLII		2 20	0–3	
Pregnane 3(α) 17 diol 20-one	CLIII		3 2	0 5	
Δ^5 Pregnene 3(β) 17 β -diol 20-one	Footnote[5]				5 3

[a] Expressed as mg /l , and excluding 17 ketosteroids and artifacts arising from the compounds listed (see footnote[5] page 602)

these side chain types The quantity normally excreted seems to be of the order of 1 mg per day One compound has been isolated which is strongly reducing and which also exhibits gluconeogenic activity, but its structure has not been elucidated Two reactions with periodic acid applicable to the appropriate urine residues, give further quantitative information concerning two other classes of metabolites, namely the 17–20 glycols (side chain types *a* and *d*, page 563), and the 20–21 ketols and glycols (side chain types *a*, *b*, *e*, and *f*, page 563) The first mentioned class which is non reducing and is contained in the neutral non ketonic alcoholic fraction of urine, yields, with periodic acid, the corresponding 17 ketosteroids, which can then be estimated colorimetrically in the usual way, while the second gives rise to formaldehyde, which may also be determined colorimetrically In Table XXII, the excretion of cortical metabolites as determined by each of these five

procedures *a* to *e*, is compared under various conditions of hypo- and hyperadrenalism

Fewer quantitative data pertain to the metabolites of group *2*, compounds *f* to *k*, mainly because of the rather elaborate chemical isolations requisite to the determination of all except pregnane-3(α),20α-diol Table XXIII shows the excretion of these products in a limited number of normal individuals and in a few cases of adrenocortical hyperfunction

1 *17-Ketosteroids*

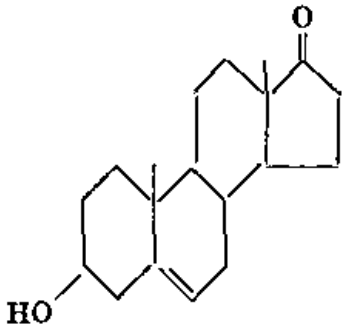
The relationship between the neutral 17 ketosteroids of urine and testicular and cortical function is more fully dealt with in Chapter XII In general, the view is accepted that these excretory products (formulated in Table XXIV) arise almost entirely from the adrenal cortex in the normal female and mainly from the cortex in the male In Addison's disease, the output of urinary 17-ketosteroids is markedly diminished According to Fraser *et al* (63) the titer is practically zero in the female and about one-third normal in the male the latter residual proportion presumably representing the contribution from the testis, in the data of Friedgood (66), however, this difference between the male and female Addisonian is less sharply defined In panhypopituitarism values as low if not lower than in Addison's disease are encountered, this is difficult to reconcile with the fact that even in the totally hypophysectomized animal some adrenal function is still retained Conversely in certain types of hyperadrenalism, particularly in cases of virilizing cortical tumors, the output may reach levels many times normal

There is very little information bearing directly on the important problem of which of the many adrenal steroids (Table III, page 557) may function as precursors of one or more of the urinary 17-ketosteroids *of cortical origin* *The ease with which the 17-hydroxylated compounds* (side chain types *a* to *d*, page 563) may be ruptured *in vitro* to the corresponding 17-ketone has long suggested that the same reaction may proceed *in vivo* In guinea pigs treated with 5 mg quantities of allopregnane-3(α),17"α" diol 20-one and of Kendall's Compound E (VIII) an increase in 17 ketosteroid output (antimony trichloride color assay) from about *0 15 to 0 45 mg per day has been observed (186), the increment accounts* for roughly 5–10% of the substance administered in each case On the other hand, in rabbits each given orally over three days 225 mg of the 3,21-diacetate of allopregnane-3(β),17"β',21 triol 20-one (the diacetate of Reichstein's Compound P, X), or given subcutaneously the same quantity of the unacetylated substance, the excretion of the corresponding 17-ketosteroid, *iso*androsterone, (CXLIII) could not be demonstrated, nor could any ketonic or non-ketonic transformation product be isolated

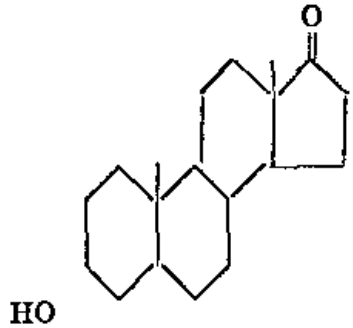
following acid hydrolysis of the urine, systematic fractionation in the usual way, and chromatographic separation (32) Neither progesterone (XXIV) nor desoxycorticosterone (XXII), both constituents of the adrenal cortex, cause any significant rise in 17-ketosteroid output

TABLE XXIV

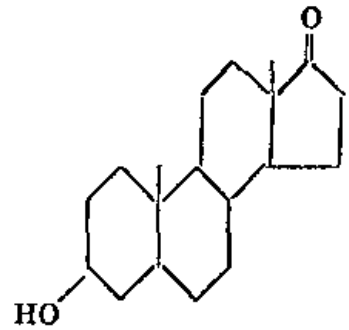
URINARY 17 KETOSTEROIDS[a]



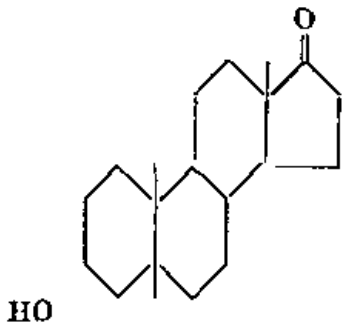
CXLI



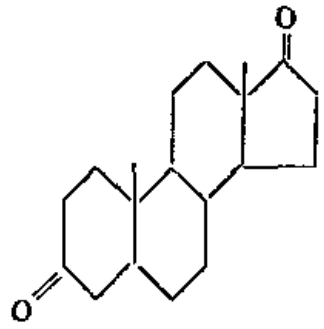
CXLII



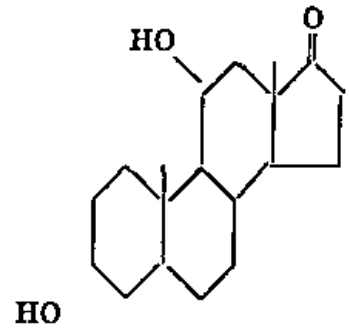
CXLIII



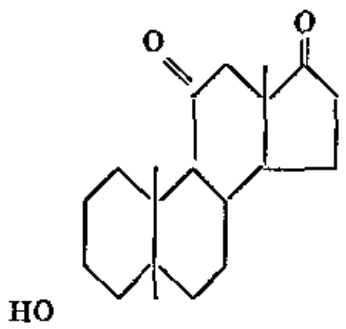
CXLIV



CXLV



CXLVI



CXLVII

[a] Exclusive of artifacts arising from the above compounds or their water-soluble conjugates

Throughout gestation when the progesterone secretion of the corpus luteum of pregnancy and of the placenta increases enormously, the urinary 17-ketosteroids remain relatively constant (44,77,79,137,187,254), also the subcutaneous or oral administration to individual male or female rabbits of up to 500 mg of progesterone does not alter significantly the

17-ketosteroid excretion (103) Desoxycorticosterone acetate administered at a dose level of 10 mg per day to both male and female Addisonians does not lead to any consequential change in titer (35), similarly, in adult male rabbits each treated with 500 mg of desoxycorticosterone acetate no significant increase in urinary 17-ketosteroids is observed (103) Following the administration of adrenocortical extracts to human subjects, no convincing alteration in urinary 17-ketosteroids has been noted, but in this connection it should be pointed out that the concentration of adrenal steroids in commercial extracts is not high on a weight/volume basis, and that the quantity given may be insufficient to reflect the metabolic pathway Stimulation (in hypopituitarism in man) of the adrenals with corticotrophin does however lead (252) to a significant increase (five to seven times) in the output of 17-ketosteroids (as determined by the Zimmermann color reaction)

2 *Cortin*

The cortin-like properties of extracts of human urine were first observed by Perla and Marmorston-Gottesman (182) in 1931 and by Grollman and Firor (78) in 1932 Ample confirmation followed, with the demonstration that the excreted principle or principles possess activity in the adrenalectomized animal with respect to (*a*) protection against histamine poisoning (182) and exposure to cold (39,262), (*b*) time survival and life maintenance (38,78), (*c*) prevention of water (220) and potassium (55) intoxication, (*d*) prolongation of the work performed by the gastrocnemius muscle (228), and (*e*) the deposition of hepatic glycogen (38,137,256,258) The chemical nature of the active metabolite(s) is not established, although Venning, Hoffman, and Browne (256) have isolated, as acetate, a crystalline ketone, melting point 234–236°, which reduces alkaline silver diamine and exhibits cold protection, also the compound promotes glycogen storage in the liver (105) As urine concentrates are corrective in both the electrolyte and carbohydrate disturbances associated with loss of adrenal function, the excretion of active substances in both the 11-desoxy- and 11-oxy-series is implied Presumably also the active metabolites are excreted in conjugated form, as extraction of urine at pH 1 0 leads to preparations with approximately twice the potency of those attained on processing at neutrality (92,137,257,258), more vigorous hydrolytic treatment, such as boiling in acid medium, destroys the active excretory products

The quantity of cortin normally eliminated per day fluctuates (±50% of the mean) from time to time in the same individual, possibly due to variation in state of activity or excitement Compared by the power to cause glycogen deposition in the liver of the adrenalectomized animal

(42,45,137,213,258) and expressed as the biological equivalent of Kendall s Compound E (VIII), normal adult females excrete from 29 to 55 μg (average, 39), with no correlation apparent with the phase of the menstrual cycle, and normal adult males, from 45 to 90 μg (average 62) (92,137,257) Exercise and activity may raise the amount excreted (137) No glycogenic activity is detectable in the urine of newborn males (1–4 days), but at 2 5 years of age the excretion has attained the adult female level, and at about 6 years the adult male value is reached (92,137,257) Subnormal to zero values are associated with hypoadrenalism (Addison's disease and panhypopituitarism), and, conversely, with cortical hyperfunction (Cushing's syndrome) and under stress (thermal trauma, the postoperative state, and gestation), values six to ten times normal are encountered (92,137,257)

Experimentally, the administration of whole adrenal cortical extract to the dog (80), monkey (41), and man (40,256,261) causes a rise in urinary cortin, the increment representing approximately 10% of the hormones contained in the original extract (effect on salt and water metabolism and cold exposure test) In the monkey, active material (cold exposure test) is still excreted following gonadectomy but not adrenalectomy (41) Stimulation of the adrenal cortex in man by the administration of pituitary adrenocorticotrophin leads to a ten to twenty-fold increase in cortin output (cold exposure test, 137)

3 *Neutral Lipide-Soluble Reducing Substances*

Talbot *et al* (250) and Heard and Sobel (91) have quantitatively standardized the reduction of cupric ion and of phosphomolybdic acid, respectively, by adrenal steroids possessing a reducing group in the molecule, and have developed colorimetric methods for the estimation of small quantities of these compounds In the first-mentioned test the reaction is given by a primary α ketol grouping (side chain types *b* and *e*, page 563), and in the second by the α-ketol and/or the α,β unsaturated 3-ketone groupings When applied to extracts of urine, general parallelism between urinary reducing power and cortical function is observed (Table XXII) By the procedure of Talbot *et al* (250), the urine is extracted without previous treatment with acid, and the colorimetric determination is made on that portion of the neutral lipide-soluble ketones which is extractable from benzene with water Heard, Sobel, and Venning (92) acidify the urine to pH 1 0 prior to extraction, an operation which, as in the estimation of urinary cortin (page 609), leads to an increase (four to five times) in the quantity of metabolites recovered, and they apply the colorimetric estimation directly to the total neutral extract By either technique the excretion of reducing substances is

approximately one-third to one-half normal in hypoadrenalism and two to ten times normal in hyperadrenalism, under stress, and in late pregnancy Normal adult values fluctuate within ±50% of the mean, by the procedure of Heard, Sobel, and Venning (92), males range from 1 1 to 2 1 (average 1 5) mg per day (calculated as desoxycorticosterone), and females from 1 0 to 2 0 (average 1 3) With increasing age in male children, the normal adult level is not attained as early in life as the output of cortin (92), by age 7, urinary reducing capacity is only 50% of the adult value, while cortin excretion has already reached the adult level (see page 610) Also, in convalescence from damage, the normal biological titer is attained more rapidly than that of the reducing substances (92) In the dog (92), extirpation of the adrenals diminishes the output by about 66%, and the intravenous injection of cortical extract causes within a few hours a sharp rise and fall, the total additional excretion representing 3 4% of the reducing capacity of the original extract, also, stimulation of the adrenals by the subcutaneous administration of whole anterior lobe extract containing adrenocorticotrophin leads to a twofold increase over normal, which persists for several days In man (hypopituitarism), corticotrophin causes a sixfold rise in the output of reducing substances (252)

It must be assumed that at least one third of the water insoluble, lipide-soluble reducing substances normally excreted are of extra-adrenal origin, as in Addison's disease and panhypopituitarism (92,250), and, following bilateral adrenalectomy in the dog (92), a minimum residual titer of at least this proportion is retained

4 *17-Ketosteroids Generated on Periodic Acid Oxidation of the Neutral Nonketonic Lipides*

As pointed out in Section III, C, 2, steroid glycols hydroxylated at C-17 and C-20 (*i e*, side chain types *a* and *d*) are readily oxidized by periodic acid to the corresponding 17-ketones Accordingly, 17-ketosteroids formed from the neutral lipides of urine by this treatment should be an index of the quantity of metabolites with these types of side chains which are excreted

The principle has been applied by Talbot and Eitingon (249) Because of the sensitivity of the tertiary 17 hydroxyl group and of conjugated alcohols to dehydration on treatment with strong acid, the conjugates are first extracted from urine with butanol, washed until neutral, and then hydrolyzed (*a*) by refluxing with barium chloride at pH 6 0, or (*b*) with rat liver enzyme, or (*c*) by refluxing for ten minutes in 15 volumes per cent hydrochloric acid, or progressively by *b* plus *a* plus *c*, *b* plus *c*, or *a* plus *c* After separation of the hydrolyzates into

ketonic and non ketonic portions with Girard reagent, colorimetric assay of the ketonic fraction and of the neutral products of the oxidation of the non-ketonic fraction with periodic acid then give, respectively, a measure of the "preformed" 17-ketosteroids and of those formed by the oxidative treatment Following hydrolytic procedures *a* or *b*, a significant quantity of 17-ketosteroids is generated from non-ketonic material on oxidation with periodic acid As none is observed after hydrolysis *c*, it follows that the precursors of the formed 17-ketosteroids are destroyed on being subjected to strong acid treatment That the precursors are excreted in conjugated state is evidenced by the significantly lower values encountered on processing the urine without any form of hydrolysis

In one case of adrenocortical hyperplasia and two cases of adrenocortical carcinoma, the daily excretion of non ketonic substances oxidizable with periodic acid to 17-ketosteroids amounted to the equivalent of 10–16 mg of the latter as compared to the excretion of about 0 4 mg by normal individuals, in the three pathological subjects, the output of preformed 17-ketosteroids was, respectively, 25, 60, and 200–375 mg (249)

Fieser, Fields, and Lieberman (57) have also observed the formation of 17-ketosteroids on periodic acid oxidation of the non-ketonic alcohols derived from commercially processed human pregnancy urine subjected to acid hydrolysis The periodic acid oxidation of 90 g of this fraction (from 467 l of urine) yielded 4 14 g (4 6%) of ketonic material which could not be crystallized after repeated chromatographic fractionation but which contained the equivalent of 0 87 g of androsterone by colorimetric assay The output of precursors is thus about 2 mg /l , a level somewhat higher than that encountered by Talbot and Eitingon (249) in normal individuals (0 4 mg per day) and suggestive of increased adrenal cortical activity during pregnancy, which is also evidenced by other criteria of cortical function (Table XXII, page 604) According to Dobriner and Lieberman (137) however, the quantity of 17-ketosteroids generated with periodic acid from the non-ketonic fraction of urine does not in general parallel the excretion of active cortin

An exploration has been carried out (57) of the possibility of separating 1 2 or 1,3-glycols from urine as their acetals with *m*-hydroxybenzaldehyde so that the condensation products could be selectively extracted from a mixture with alkali While acetal formation readily took place in model experiments with Δ^4-pregnen-17"α,' 20,21-triol-3-one and Δ^5-pregnene-3(β),20,21-triol, only 0 and 20%, respectively, of the original steroid could be recovered from the acetals by acid hydrolysis, presumably due to the high sensitivity of α glycols to dehydration

At least one non-ketonic metabolite has been isolated which gives rise

to a 17-ketosteroid on oxidation with periodic acid, namely, pregnane-3(α),17,20-triol (CLII). In all probability the 3(β)-hydroxy epimer of CLII constitutes another

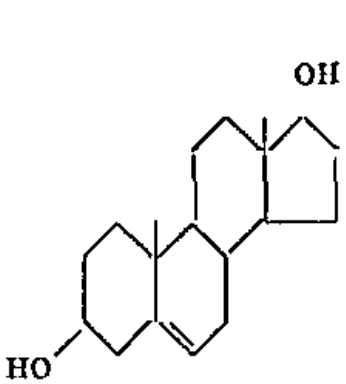
5 *Formaldehyde Generated on Periodic Acid Oxidation of Neutral Lipides*

Cleavage of 20–21-α-ketols or glycols (side chain types *a*, *b*, *e*, and *f*, page 563) releases formaldehyde, the estimation of which may serve to indicate the quantity of metabolites possessing these types of side chain which are excreted

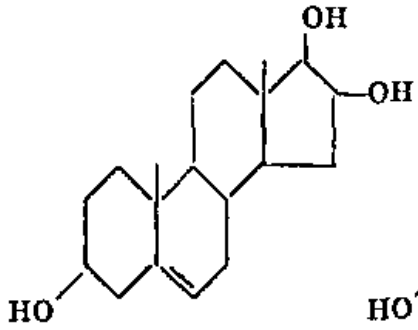
In a preliminary communication, Loewenstein, Corcoran, and Page (136) have reported the formaldehyde titer of the periodic-acid oxidation products of the neutral lipides extracted from acidified urine Normally the daily excretion amounts to the equivalent of 0 5 to 0 8 mg of dehydrocorticosterone (XVI), in a case of Addison's disease and of Cushing's syndrome, outputs of 0 15 and 21 mg, respectively, were observed

TABLE XXV

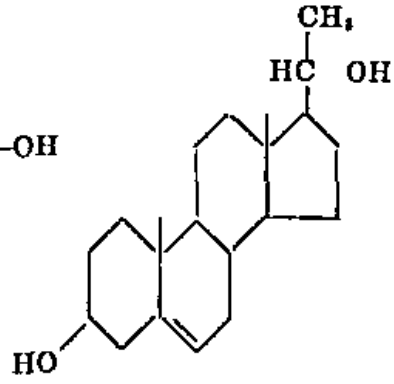
CORTICAL METABOLITES OTHER THAN 17-KETOSTEROIDS[a]



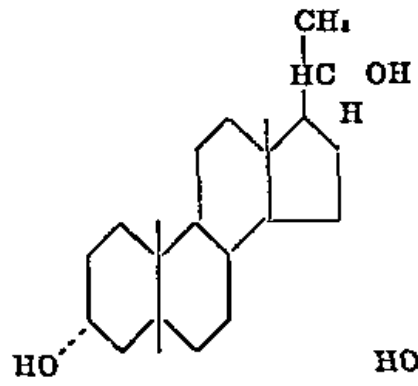
CXLVIII



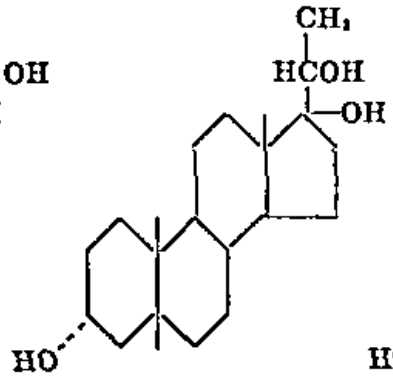
CXLIX



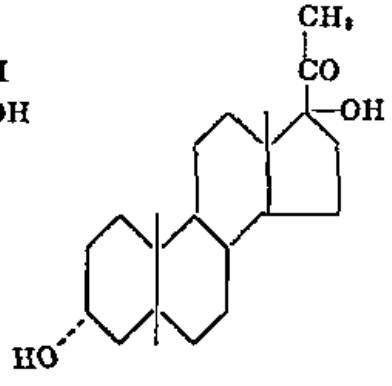
CL



CLI



CLII



CLIII

[a] See footnote[a]

6 Δ^5-*Androstene-3(β),17(α)-diol*

Large quantities (8–48 mg /l of Δ^5-androstene-3(β),17(α)-diol (CXLVIII, page 613) have been isolated by Hirschmann and Hirschmann (99) and by Schiller *et al* (221) in cases of adrenocortical carcinoma in either sex, smaller quantities occur in the urine of female patients with adrenal tumor and little or none in cases of hyperplasia (164), the compound has not been met with in fractionation of normal urine That CXLVIII may arise *in vivo* from dehydro*iso*androsterone (CXLI, page 608) is clearly illustrated from the isolation by Mason and Kepler (163) of 6 5 mg of the diol from the urine of a subject with impaired cortical function given 1090 mg of CXLI (as acetate), the reaction is reversible, as in the guinea pig the administration of the diol leads to the excretion of the ketone (173) In the rabbit however, the analagous metabolite of dehydro*iso*androsterone (CXLI) is the 17(β) epimer of CXLVIII (103) It is interesting to note that the same species difference in the stereochemical configuration of the 17-hydroxyl group obtains in the estrogen series, where, in man, α-estradiol arises from estrone, while in the rabbit β-estradiol is the principal excretory product (Chapter X)

7 Δ^5-*Androstene-3(β),16,17-triol*

Δ^5-Androstene-3(β),16,17-triol (CXLIX), is present in the urine of normal men and non-pregnant women in concentrations of the order of 0 1 mg /l (Marrian and Butler, 158) That it arises from the adrenal cortex is strongly suggested by the much higher output (*ca* 20 mg or more per l) in cases of adrenal carcinoma or tumor (Hirschmann, 98, Mason and Kepler, 164) Hirschmann (98) offers the attractive explanation that the 16,17-glycol CXLIX is derived from dehydro*iso*androsterone (CXLI) through the same series of metabolic reactions believed to be associated with conversion of an analagous 17-ketone, estrone, to one of its 16,17-glycols, estriol Consistent with this view are the facts that all of the many attempts to identify dehydro*iso*androsterone as a metabolite of testosterone have met with failure, and that the excretion of excessive amounts of dehydro*iso*androsterone (and of 3(β) hydroxy-17-ketosteroids in general) is associated with adrenocortical tumor or carcinoma The extent of the conversion is seemingly small, however, as Mason and Kepler (165) were able to isolate only 3 mg of the triol from one of two Addisonians maintained on desoxycorticosterone acetate and given 1090 mg of dehydro*iso*androsterone (as acetate), and, in normal rabbits given 4 g of the ketone, no significant quantity of the urinary triol was excreted (103)

It is not implied however, that the metabolism of dehydro*iso*andro-

sterone supplied exogenously to normal individuals or a subject with hypopituitarism need follow the same pathway as that elaborated in a carcinomatous adrenal cortex

8 Δ^5-*Pregnene-3*(β),*20*α-*diol*

Large quantities (35 mg /l) of Δ^5-pregnene-3(β),20α-diol (CL), have been isolated (as mono- and di-acetates) from the urine of patients with adrenocortical carcinoma by Hirschmann and Hirschmann (99) and by Schiller *et al* (221), it also occurs in equine pregnancy urine (149) *As the compound has escaped detection in the numerous explorations* of normal urine, the physiological excretion, if any must be exceedingly small, and the excess in the above instances is assumed to arise from the adrenal cortex

9 *Pregnane-3*(α) *20*α-*diol*

The excretion of pregnane-3(α) 20α-diol (CLI), as 3-monoglucuronide, which in the human represents the chief urinary transformation product of progesterone (see Chapter XI), provides a measure of both luteal and adrenocortical function The output of approximately 0 1 mg per day observed in the pre-ovulatory phase of the normal menstrual cycle (255), in the ovariectomized woman (97), and in man (48,86) reflects the normal contribution from the adrenal cortex, while much larger amounts arise from the corpus luteum and placenta, as evidenced by the daily excretion of quantities approaching 10 mg in the post-ovulatory phase of the normal menstrual cycle and 100 mg in late pregnancy The relatively small amount, presumably of adrenal origin, excreted in the absence of a functioning corpus luteum or placenta is not measurable by the gravimetric method of Venning (253) for the estimation of pregnanediol glucuronide, in these instances, roughly quantitative values are arrived at by the isolation of the free pregnanediol after acid hydrolysis of the conjugate in urine In virilism and pseudohermaphroditism in the female due to adrenocortical tumor or carcinoma, 10–20 mg daily of pregnanediol are excreted, but in cases of simple hyperplasia elevated values are not consistently observed (25,58,164,218,248 259)

The pregnanediol of adrenal origin may arise from progesterone (XXIV), or desoxycorticosterone (XXII) or both XXIV has been isolated (4,6) from cortical tissue and its conversion *in vivo* to pregnanediol is conclusively established (Chapter XI) That desoxycorticosterone is, in part, excreted as pregnanediol was first indicated by Cuyler, Ashley, and Hamblen (34), who claimed a rise in the pregnanediol glucuronide titer of normal men treated with XXII While the view was not credited in later communications from the same laboratory

(35), substantiation followed with the experimental demonstrations by Westphal (263) and Hoffman, Kazmin, and Browne (104) that 7–15% of the desoxycorticosterone administered to rabbits is eliminated as pregnanediol glucuronide Dorfman and collaborators (60,107) have observed a 3% conversion of desoxycorticosterone to pregnanediol in the chimpanzee and man Conceivably also, other adrenal steroids may give rise to pregnanediol, although there is no direct supporting evidence, if such is not the case, however, the hundredfold increase in pregnanediol excretion in cases of adrenal carcinoma must be attributed to colossal over-production of progesterone and/or desoxycorticosterone

10 *Pregnane-3(α),17,20 triol*

Large quantities (*ca* 20 mg /l) of pregnane-3(α),17,20 triol (CLII), have been isolated by Butler and Marrian (25,26,151) from the urine of women with hyperplastic adrenals and symptoms of virilism together with much smaller amounts (*ca* 0 3 mg /l) of a second triol, $C_{21}H_{36}O_3$ the structure of which is not fully established but which appears to be the digitonin-precipitable 3(β)-hydroxy epimer of CLII The urinary triol CLII represents a stereoisomer of the adrenal compounds J and O (XVIII and XIX), of Reichstein's series, it is epimeric at C-5, bears at C-20 the hydroxyl group in either of the two orientations observed in Compounds J and O (which differ from each other only in this regard), and presumably possesses at C-17 the stable configuration of the adrenal steroids Neither substance has been encountered in the processing of normal urine by the usual methods (which incorporate acid hydrolysis), but it is perhaps highly significant that the isolation of large amounts of these acid sensitive 17 hydroxy steroids was accomplished from urine specimens which had not been subjected to hydrolytic treatment but which may have been exposed to the action of bacterial enzymes In the experience of Mason and Kepler (164), the excretion of CLII (which they observed in one instance in the form of a glucuronidate) is generally confined to patients with cortical hyperplasia and is not frequently encountered in cases of adrenal tumor CLII is oxidized by periodic acid to etiocholan-3(α)-ol 17 one (CXLIV) and acetaldehyde, accordingly it is included in the estimates of cortical metabolites from which 17-ketosteroids are generated on treatment with periodic acid

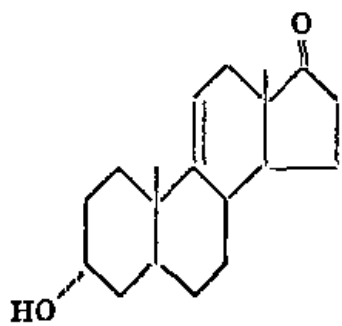
11 *Pregnane-3(α),17-diol-20-one*

Lieberman and Dobriner (130) have separated appreciable quantities of pregnane-3(α),17 diol 20-one (17 hydroxypregnanolone, CLIII) from the urine of subjects with adrenal hyperplasia (3 2 mg /l), adrenal

tumor (0 5 mg /l), cryptorchidism (5 8 mg /l), and a eunuch given testosterone (0 007 mg /l) In all cases except that of the cryptorchid the urine was acidified and boiled prior to extraction, in this case, which afforded the highest yield, heat treatment was not applied Careful examination of large volumes of the urine of normal individuals and of pregnant women failed to reveal the excretion of CLIII The compound is stereoisomeric with Reichstein's Compound L (XX) from cortical extract, differing in configuration about C-3 and C-5, as in the case of the urinary triol CLII, it is highly probable that the 17-hydroxyl group is oriented in the stable configuration of the adrenal steroids That it arises from the adrenal is strongly indicated from (*a*) the nature of the pathological states in which its excretion is observed, and (*b*) the presence in the molecule of the tertiary 17-hydroxyl group, which is distinctive of the adrenal steroids Although CLIII possesses an α-ketol grouping, the hydroxyl function of the latter is tertiary, accordingly this metabolite is non reducing, stable to periodic acid, and is not estimated by procedures *c* to *e*

12 *Urinary Steroids Oxygenated at C-11*

The failure to recognize any appreciable quantity of 11-oxygenated compounds among the urinary steroids has long been conspicuous and quite irreconcilable with the fact that at least 10 mg of 11-desoxy adrenal metabolites are excreted every 24 hours It is now apparent that the 11(β) hydroxyl group may be largely eliminated by dehydration (see Table VI, page 568) in the course of the acid treatment usually applied to the hydrolysis of the water-soluble conjugates in urine Mason (161) and Mason and Kepler (164) isolated 11(β) hydroxy androsterone (CXLVI) from the acid-hydrolyzed urine of normal men (0 3 mg /l) and of subjects with adrenal cortical tumor or hyperplasia (1 5 to 2 1 mg /l), and showed that the 11 hydroxyl group, as in the natural adrenal steroids, is sensitive to acid treatment which leads to an androsten-3(α)-ol-17-one (CLIV) in which the ethylenic linkage is tentatively

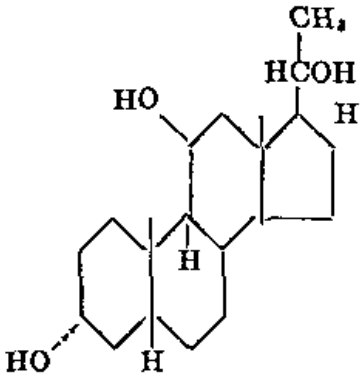


CLIV

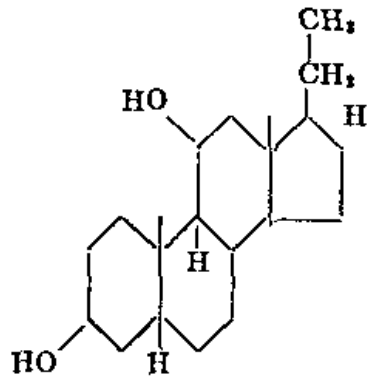
assigned the 9,11 position (the 11,12 position or a mixture of the two is not excluded) From a case of adrenal virilism, Miller, Dorfman, and Sevringhaus (174) isolated the same 11-hydroxy androsterone (CXLVI) in much larger amount (10 5 mg /l) from urine not subjected to hydrolytic treatment, and showed that its dehydration product (CLIV) was identical with the androsten-3(α) ol-17-one previously separated (4 8 mg /l) by Dorfman, Schiller, and Sevringhaus (43) from acid-hydrolyzed urine of the same subject, and probably identical with that earlier obtained by Wolfe, Fieser, and Friedgood (270), also from the acid-treated urine of a girl with cortical tumor It is thus apparent that the unsaturated compound CLIV is an artifact, and that 11-hydroxy-androsterone represents a normal adrenal metabolite

More recently a second 11-oxygenated metabolite has been isolated from the urine of normal men and women (1–2 mg /l) by Lieberman and Dobriner (131), who proved the structure to be etiocholan-3(α)-ol-11,17-dione (CXLVII)

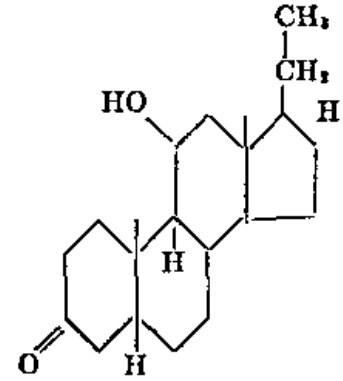
An interesting series of C_{21} metabolites, probably oxygenated at C-11, have been separated from equine pregnancy urine by Marker and collaborators (142,144,148,153) The parent hydrocarbon, $C_{21}H_{36}$, is not identical with pregnane or allopregnane and is assigned the 9-*iso*pregnane structure (urane) The three derivatives isolated, a uranetriol (CLV), a uranediol (CLVI), and a uranolone (CLVII), are formulated as shown below



CLV



CLVI



CLVII

a non-reactive hydroxyl group is placed at C-11 in all cases Marker (138) suggests that such transformation products may arise on saturation of an ethylenic linkage issuing from C-9 While the occurrence in mare pregnancy urine of a compound identical in its physical properties and in those of its derivatives with uranediol (CLVI) has been confirmed (3,117), strict chemical proof of the 9-*iso*pregnane structure and of substitution at C-11 remains to be furnished

In addition to the excretory products listed above, several compounds

of unestablished chemical structure have been isolated in crystalline state from the urine of human subjects with adrenal hyperplasia, tumor or carcinoma (37,99,164,165) These conditions of excretion clearly imply a close metabolic relationship to the adrenal corticosteroids, but as the compounds are characterized only by physical constants and empirical formulae, appreciation of the metabolic significance must await the full elucidation of chemical constitution

13 *Urinary Steroids Probably of Cortical Origin*

The urinary steroids listed in Table XXVI have been separated, as indicated, mainly from the urines of normal and pregnant humans and domestic animals Thus the physiological conditions under which the excretion of these metabolites has been noted neither suggests nor denies cortical origin, but there is indirect evidence that some, if not all, of these excretory products arise, at least in part, from the adrenal cortex

The pregnane and allopregnane derivatives 2 to 10 in the table are all partial or complete saturation products of progesterone (XXIV), and, as such, may conceivably arise *in vivo* from progesterone of ovarian, placental, or adrenal origin It is significant, however, that none of the possible reduction products of progesterone other than pregnane-3(α),20α-diol (CLI) has actually been isolated in excessive amount following the administration of the corpus luteum hormone to man or experimental animals, in spite of systematic searches (85,263) As it has been conclusively proven (page 616) that desoxycorticosterone (XXII) acetate gives rise to pregnane-3(α),20α-diol, it follows that intermediates in this conversion may be excreted, further, this demonstration of the reduction *in vivo* of the 21-alcoholic group to the 21-methyl group renders plausible a similar metabolic pathway in the cases of other adrenal steroids Also it is to be noted that the isolation of the compounds listed in Table XXVI has been accomplished almost exclusively from the urine of pregnancy, during which period the adrenal is known to be hyperactive with respect to excretion of other cortical metabolites, namely cortin and reducing substances (Table XXII, page 604), only pregnane-3(α),20α-diol and allopregnane-3(α),20α-diol have been shown to occur in human non-pregnancy urine, the latter in quantity approximately one sixth that present in pregnancy urine (156) The observations of Marker *et al* (156) concerning the distribution of pregnane-3(α),20α-diol, allopregnane-3(α),20α-diol and allopregnane-3(β),20α-diol in the urine of the bull and the pregnant mare are intriguing, with respect to each of these three compounds, the output of the male is twice that of the pregnant female, which points to the testis (in addition to ovary, placenta, and adrenal) as a very important source Further suggestion

TABLE XXVI

URINARY STEROIDS POSSIBLY OF CORTICAL ORIGIN[a]

	Steroid	Urinary source	Ref
1	Allopregnane 3(α),16,20 triol	Mare pregnancy	83,84,145,154, 155,176 236
2	Allopregnane 3(α),20α-diol	Human pregnancy	82
		Cow pregnancy	139
		Mare pregnancy	141
		Human non-pregnancy	156
		Adult bull	156
3	Allopregnane-3(β),20α-diol	Human pregnancy	150
		Cow pregnancy	139
		Mare pregnancy	150
		Adult bull	156
4	Allopregnane-3(β),20β-diol	Mare pregnancy	3
5	Pregnan 3(α)-ol 20-one	Human pregnancy	140,180
		Sow pregnancy	152
		Human neoplasm	37
6	Allopregnan 3(α)-ol 20-one	Human pregnancy	143
		Human neoplasm	37
7	Allopregnan 3(β)-ol 20-one	Human pregnancy	181
		Mare pregnancy	89,148,178
		Sow pregnancy	152
8	Pregnane-3,20-dione	Mare pregnancy	148
9	Allopregnane-3,20-dione	Mare pregnancy	148
		Human neoplasm	37
10	Pregnan 3(α)-ol	Human pregnancy	146
11	Androstan 3(β)-ol x-one	Mare pregnancy	89,90,178

[a] Etiocholane-3(α),17(α) diol is frequently listed (187,227) as a constituent of normal male human urine While this compound was isolated by Butenandt and collaborators (16,24) from human male urine which had been subjected to the reductive action of aluminum amalgam, they were unable to confirm its presence in urine not so treated as the original investigators point out, the diol may well represent an artifact arising from the saturation of a ketone—such as etiocholan 3(α)-ol-17-one (CXLIV) Etiocholane 3(α),17(α)-diol has however been isolated from the urine of rabbits and normal men treated with testosterone (103)

that these reduction products of progesterone may arise in part from the adrenal cortex is afforded by the isolations of Dobriner *et al* (37) of allopregnane-3,20-dione, allopregnan-3(α)-ol-20-one and pregnan-3(α)-ol-20-one from the urine of patients with neoplastic disease, and by the isolation by Mason and Kepler (164) from the urine of women with adrenal hyperplasia of a substance agreeing in physical properties with pregnane-3(β),20(α)-diol; none of these compounds has been reported in normal urine

No positive information is forthcoming concerning the origin of the

remaining two urinary steroids listed in Table XXVI as their isolation has been effected only from mare pregnancy urine The same source has also provided a number of steroids of undetermined chemical structure which may well represent adrenal metabolites among these may be mentioned a keto-lactone $C_{19}H_{26}O_3$ (84 112) a compound possibly identical with Δ^{16} allopregnenol-3(β)-ol 20 one which is excreted as its sulfuric acid ester (117 118) and two unknown isomers of pregnanolone and an isomer of androsterone (178) conceivable additional adrenal metabolites are an allopregnanetriol and an allopregnanetetrol which occur in stallion urine (147) but in which the position of the hydroxyl groups has not been ascertained

Excessive quantities of estrogen as determined by bioassay are excreted in certain cases of adrenal tumor (15 61 62 235) and estrone has recently been isolated by Mason and Kepler (164) from the urine of one such subject α-estradiol and estriol could not be identified The additional estrogen is probably of cortical origin as the quantity obtained (*ca* 1 5 mg /l) far exceeds the normal output in the non pregnant state (*ca* 0 05 mg /l) and presumably it arises through overproduction in the presence of the lesion of estrone which has been identified (5) as a constituent of normal cortical tissue

B ANABOLISM

The large quantity of steroid hormone metabolites excreted per day (up to *ca* 30 mg in the non pregnant state and up to *ca* 200 mg near parturition—Section VII A) stands in sharp contrast to the extremely minute quantities (of the order of 1 part in 40 000–15 000 000) of active hormones which may be demonstrated to be present in the appropriate secretory organs (gonads adrenal and placenta) of a single individual and has long indicated rapid syntheses of the humoral agents from anabolites of low molecular weight or a rapid turnover from a more abundant immediate precursor Evidence is now accumulating in direct support of the postulate that cholesterol which constitutes about 4% of adrenal tissue functions *in vivo* as a precursor of the bile acids and steroid hormones This information gained mainly through the application of the tracer elements deuterium and C^{13} tends to invalidate earlier hypotheses (207) that the highly oxygenated adrenal steroids in particular may arise from a condensation of 3-carbon triose residues (*e g* dihydroxyacetone glyceraldehyde *etc*) and subsequent reduction

That cholesterol may be synthesized in the animal organism has been recognized since 1933 when Schoenheimer and Breusch (222) clearly demonstrated in mice an accumulation of cholesterol over and above the

restricted dietary intake, conversely, with the ingestion of excessive amounts of cholesterol, much disappeared from the body More convincing proof of the synthesis of cholesterol followed the introduction of isotopic hydrogen as a tracer element The utilization of acetic acid by yeast in the formation of sterols was shown in 1937 by Sonderhoff and Thomas (237), and, in 1942, Bloch and Rittenberg (9) found that acetic acid, fed to mice as sodium deuterioacetate, was incorporated into both the side chain and ring system of the cholesterol molecule It is estimated that at least half the hydrogen atoms of cholesterol are derived from the acetate (11) Further substantiation of the acetic-acid–cholesterol conversion came with the use of sodium acetate in which the methyl group contained 70% deuterium and the carboxyl group, excess of the stable heavy isotope of carbon, C^{13}, administered to mice, the isolated cholesterol contained sufficient H^2 and C^{13} to indicate the assimilation of both carbon atoms of the acetic acid into the sterol (214) Propionic, butyric, and succinic acids failed to give rise to the sterol (9), which indirectly excludes pyruvic and acetoacetic acids as intermediates in the synthesis and which bears out the thesis earlier (1937) enunciated by Rittenberg and Schoenheimer (215) that the utilization of the higher fatty acids in the synthesis of the sterol molecule is quite improbable As concerns the breakdown *in vivo* of cholesterol, the administration of deuteriocholesterol (10) to dogs (8) and to a woman in the eighth month of gestation (7) led to the excretion, in the bile and urine, respectively, of deuterium-rich cholic acid and pregnane-3(α),20α-diol These observations clearly suggest that the C_{27} sterol is catabolized in the body to the C_{24} bile acid and to the C_{21} excretory product of progesterone, in the latter conversion it is inferred that the reactions proceed through the luteal hormone itself In the demonstration of these anabolic and catabolic reactions in which cholesterol participates, it has been shown in all cases that the concentration of deuterium or C^{13} in the product, relative to that in the starting material, is sufficiently high as to render improbable deuterium exchange reactions or syntheses involving heavy water or carbon dioxide resulting from the total combustion of the isotopic substance under examination

Good presumptive evidence that cholesterol functions as an immediate precursor of the adrenocortical hormones is provided by the disappearance of large quantities of this sterol from the hyperactive gland (*i e*, during stress, or following stimulation from corticotrophin) Commensurate with the utilization of the sterol, there is a marked decline in ascorbic acid content, which strongly suggests that the vitamin is intimately associated with the mechanism of the supposed conversion of cholesterol to the corticosteroids The physiological significance of the

cholesterol–ascorbic-acid–cortical-hormone relationships is considered in detail and with complete bibliography in the chapter by R L Noble, Vol II

REFERENCES

1 Alther, H B, and Reichstein, T *Helv Chim Acta* **25**, 805 (1942)
2 Bachmann, W E, Cole, W, and Wilds, A L *J Am Chem Soc* **62**, 824 (1940)
3 Bauld, W S, and Heard, R D H Unpublished observation
4 Beall, D *Biochem J* **32**, 1957 (1938)
5 Beall, D *Nature* **144**, 76 (1939), *Endocrinology* **2**, 81 (1940)
6 Beall, D, and Reichstein, T *Nature* **142**, 479 (1938)
7 Bloch, K. *J Biol Chem* **157**, 661 (1945)
8 Bloch, K, Berg, B N, and Rittenberg, D *J Biol Chem* **149**, 511 (1943)
9 Bloch, K, and Rittenberg, D *ibid* **143**, 297 (1942), **145**, 625 (1942)
10 Bloch, K, and Rittenberg, D *ibid* **149**, 505 (1943)
11 Bloch, K, and Rittenberg D *ibid* **159**, 45 (1945)
12 Brink, N G, Clark, D M, and Wallis E S *ibid* **162**, 695 (1946)
13 Bruzzone S, Borel, H, and Schwartz J *Endocrinology* **39**, 194 (1946)
14 Burckhardt, V, and Reichstein, T *Helv Chim Acta* **25**, 821 (1942)
15 Burrows, H, Cook, J W, Roe, E M F, and Warren, F L *Biochem J* **31**, 950 (1937)
16 Butenandt, A *Angew Chem* **45**, 655 (1932)
17 Butenandt, A, and Fleischer, G *Ber* **70**, 96 (1937)
18 Butenandt, A, and Mamoli, L *ibid* **68**, 1847 (1935)
19 Butenandt, A, and Peters, D *ibid* **71**, 2688 (1938)
20 Butenandt, A, and Poschmann, L *ibid* **77B**, 392 (1944)
21 Butenandt, A, and Poschmann, L *ibid* **77B**, 394 (1944)
22 Butenandt, A, and Schmidt-Thomé, J *ibid* **72**, 182 (1939)
23 Butenandt, A, Schmidt-Thomé, J, and Paul H *ibid* **72**, 1112 (1939)
24 Butenandt, A., Tschernıng, K, and Dannenberg, H *Z physiol Chem* **248**, 205 (1937)
25 Butler, G C, and Marrian, G F *J Biol Chem* **119**, 565 (1937)
26 Butler, G C, and Marrian G F *ibid* **124**, 237 (1938)
27 Callow, R K *Ann Rept Chem Soc* **35**, 281 (1938)
28 Cartland, G F, and Kuizenga, M H *Am J Physiol* **117**, 678 (1936)
29 Chakravorty, P N, and Wallis, E S *J Am Chem Soc* **62**, 318 (1940)
30 Clark, D M, Brink, N G, and Wallis E S *J Bio Chem* **162**, 663 (1946)
31 Cleghorn R K *Endocrinology* **32** 105 (1943)
32 Cohen, J, and Heard, R D H Unpublished observations
33 Crowfoot, D *Vitamins and Hormones* **2**, 409 (1946)
34 Cuyler, W K, Ashley, C, and Hamblen, E C *Endocrinology* **27**, 177 (1940)
35 Cuyler, W K, Hirst, D V, Powers, J M, and Hamblen, E C *J Clin Endocrinol* **2**, 373 (1942)
36 Dimroth, O, and Schweizer, R. *Ber* **56**, 1375 (1923)
37 Dobriner, K, Rhoads C P, Lieberman S, Hill, B R, and Fieser, L F *Science* **99**, 494 (1944)
38 Dorfman, R. I, and Horwitt, B N *Federation Proc* **2**, 60 (1943)
39 Dorfman, R. I, Horwitt, B N, and Fish, W R *Science* **96**, 496 (1942)
40 Dorfman, R I, Horwitt, B N, and Shipley, R A *Endocrinology* **35**, 121 (1944)

41 Dorfman, R I , Horwitt, B N , Shipley, R A , and Abbott, W E *ibid* **36**, 15 (1944)
42 Dorfman, R I , Ross, E , and Shipley, R A *ibid* **38**, 178 (1946)
43 Dorfman, R I , Schiller, S , and Sevringhaus, E L *ibid* **37**, 262 (1945)
44 Dorfman, R I , and Van Wagenen, G *Surg Gynecol Obstet* **73**, 545 (1941)
45 Eggleston, N M , Johnston, B J , and Dobriner, K *Endocrinology* **38**, 197 (1946)
46 Ehrenstein, M *J Org Chem* **6**, 626 (1941)
47 Ehrenstein, M *ibid* **9**, 435 (1944)
48 Engel, L L , Thorn, G W , and Lewis, R A *J Biol Chem* **137**, 205 (1941)
49 von Euw, J , Lardon, A , and Reichstein, T *Helv Chim Acta* **27**, 821 (1944)
50 von Euw, J , Lardon, A , and Reichstein, T *ibid* **27**, 1287 (1944)
51 von Euw, J , and Reichstein, T *Helv Chim Acta* **23**, 1114, 1258 (1940)
52 von Euw, J , and Reichstein, T *ibid* **24**, 1140 (1941)
53 von Euw, J , and Reichstein, T *ibid* **30**, 205 (1947)
54 Everse, J W R , and de Fremery, P *Acta Brevia Neerland Physiol Pharmacol Microbiol* **2**, 152 (1932)
55 Feil, M L , and Dorfman, R I *Endocrinology* **37**, 437 (1945)
56 Fieser, L F The Chemistry of Natural Products Related to Phenanthrene Reinhold, New York, 1937
57 Fieser, L F , Fields, M , and Lieberman, S *J Biol Chem* **156**, 191 (1944)
58 Finkler, R S *J Clin Endocrinol* **1**, 151 (1941)
59 Fish W R , Horwitt, B N , and Dorfman, R I *Science* **97**, 227 (1943)
60 Forbes, A P Macy Foundation Reports, Conference on Bone and Wound Healing, Third Meeting, New York, March 12–13 (1943)
61 Frank, R T *Proc Soc Exptl Biol Med* **31**, 1204 (1934)
62 Frank, R T *J Am Med Assoc* **109**, 1121 (1937)
63 Fraser, R W , Forbes, A P , Albright, F , Sulkowitch, H , and Reifenstein, E C , Jr *J Clin Endocrinol* **1**, 234 (1941)
64 Freeman, W , Pincus G , and Glover, E D *Proc Assoc Internal Secretions* Chicago Meeting (1944)
65 de Fremery, P , Laqueur, E , Reichstein, T , Spanhoff, R W , and Uyldert, I E *Nature* **139**, 26 (1937)
66 Friedgood, H B , in Moulton, F R The Chemistry and Physiology of Hormones Science Press, Washington, 1944, p 195
67 Fuchs H G , and Reichstein, T *Helv Chim Acta* **26**, 511 (1943)
68 Gallagher, T F In Recent Progress in Hormone Research Academic Press, New York, 1946, p 83
69 Gallagher, T F , and Hollander, V P *J Biol Chem* **162**, 533 (1946)
70 Gallagher, T F , and Long, W P *ibid* **162**, 495 (1946)
71 Gallagher, T F , and Long, W P *ibid* **162**, 521 (1946)
72 Gaunt, R , and Hays, H W *Science* **88**, 576 (1938)
73 Gaunt, R , Nelson, W O , and Loomis, E *Proc Soc Exptl Biol Med* **39**, 319 1938)
74 Giacomello, G *Gazz chim ital* **69**, 790 (1939)
75 Girard, A , and Sandulesco, G *Helv Chim Acta* **19**, 1095 (1936)
76 Gratton, J F , and Jensen, H *J Biol Chem* **135**, 511 (1940)
77 Greene, R R , Burrill, M W , and Thomson, D M *Endocrinology* **27**, 469 (1940)
78 Grollman, A , and Firor, W M *Proc Soc Exptl Biol Med* **30**, 669 (1932–33)
79 Hain, A M *Quart J Exptl Physiol* **29**, 139 (1939)

80 Harrop, G A, and Thorn, G W *J Exptl Med* **65**, 757 (1937)
81 Hartman, F A, and Brownell, K A *Science* **72**, 76 (1930)
82 Hartmann, M, and Locker, F *Helv Chim Acta* **18**, 160 (1935)
83 Haslewood, G A D, Marrian, G F, and Smith, E R *Biochem J* **28**, 1316 (1934)
84 Heard, R D H *J Am Chem Soc* **60**, 493 (1938)
85 Heard, R D H, Bauld, W S, and Hoffman, M M *J Biol Chem* **141**, 709 (1941)
86 Heard, R D H, and Hoffman, M M *ibid* **141**, 329 (1941)
87 Heard, R. D H, Ingle, D J, and Venning, E H Unpublished observations
88 Heard, R D H, Lawrence, D K, and Wasson, B K Unpublished observations
89 Heard, R D H, and McKay, A F *J Biol Chem* **131**, 371 (1939)
90 Heard, R D H., and McKay, A F *ibid* **165**, 677 (1946)
91. Heard, R. D H, and Sobel, H *ibid* **165**, 687 (1946)
92 Heard, R D H, Sobel, H, and Venning E H *ibid* **165**, 699 (1946)
93 Hegner, P, and Reichstein, T *Helv Chim Acta* **26**, 715 (1943)
94 Hegner, P, and Reichstein, T *ibid* **26**, 721 (1943)
95 Hicks, E M, Berg, C J, and Wallis, E S *J Biol Chem* **162**, 633 (1946)
96 Hicks, E M, and Wallis, E S *ibid* **162**, 641 (1946)
97 Hirschmann, H *ibid* **136**, 483 (1940)
98 Hirschmann, H *ibid* **150**, 363 (1943)
99 Hirschmann, H, and Hirschmann, F B *ibid* **157**, 601 (1945)
100 Hirschmann, H, and Hirschmann, F B *ibid* **167**, 7 (1947)
101 Hirschmann H, and Wintersteiner, O *ibid* **126**, 737 (1938)
102 Hoehn, W M, and Mason, H L *J Am Chem Soc* **60**, 1493 (1938)
103 Hoffman, M M Unpublished data
104 Hoffman, M M, Kazmin, V, and Browne, J S L *J Biol Chem* **147**, 259 (1943)
105 Hoffman, M M, and Venning, E H Unpublished data
106 Hollander, V P, and Gallagher, T F *J Biol Chem* **162**, 549 (1946)
107 Horwitt, B N, Dorfman, R I, Shipley, R A, and Fish W R *ibid* **155**, 213 (1944)
108 Ingle, D J *Am J Physiol* **116**, 622 (1936)
109 Ingle, D J *Proc Soc Exptl Biol Med* **44**, 450 (1940)
110 Ingle, D J *Endocrinology* **26**, 472 (1940)
111 Ingle, D J *ibid* **34**, 191 (1944)
112 Jacobs, J D, and Laqueur, E *Rec trav chim* **58**, 77 (1939)
113 Jacobsen, R P *J Am Chem Soc* **66**, 662 (1944)
114 Kendall, E C *J Am Med Assoc* **116**, 239 (1941)
115 Kendall, E C *Arch Path* **32**, 474 (1941)
116 Kendall, E C In Recent Progress in Hormone Research I Academic Press, New York, 1946, p 65
117 Klyne, W *Biochem J* **40**, *Proc* lv (1946)
118 Klyne, W, and Marrian, G F *ibid* **39**, *Proc* xlv (1945)
119 Koechlin, B, and Reichstein, T *Helv Chim Acta* **25**, 918 (1942)
120 Kuizenga, M H, in Moulton, F R The Chemistry and Physiology of Hormones Science Press, Washington, 1944, p 57
121 Kuizenga, M H, and Cartland, G F *Endocrinology* **24**, 526 (1939)
122 Kuizenga, M H, Wick, A N, Ingle, D J, Nelson, J W, and Cartland, G F *J. Biol Chem* **147**, 561 (1943)

123 Lardon, A , Grandjean, P , Press, J , Reich, H , and Reichstein, T. *Helv Chim Acta* **25**, 1444 (1942)
124 Lardon, A , and Reichstein, T *ibid* **26**, 586 (1943)
125 Lardon, A , and Reichstein, T *ibid* **26**, 607 (1943)
126 Lardon, A , and Reichstein, T *ibid* **26**, 705 (1943)
127 Lardon, A , and Reichstein, T *ibid* **26**, 747 (1943)
128 Lardon, A , and Reichstein, T *ibid* **27**, 713 (1944)
129 Lardon, A , and Reichstein, T *ibid* **28**, 1420 (1945)
130 Lieberman, S , and Dobriner, K *J Biol Chem* **161**, 269 (1945)
131 Lieberman, S , and Dobriner, K *ibid* **166**, 773 (1946)
132 Linnell, W H , and Roushdi, I M *Nature* **148**, 595 (1941)
133 Long, C N H , Katzin, B , and Fry, E L *Endocrinology* **26**, 309 (1940)
134 Long, W P , and Gallagher, T F *J Biol Chem* **162**, 511 (1946)
135 Longwell, B B , and Wintersteiner, O *J Am Chem Soc* **62**, 200 (1940)
136 Lowenstein B E , Corcoran, A C , and Page, I H *Endocrinology* **39**, 82 (1946)
137 Macy, Josiah, Jr Foundation Repts, Conference on Metabolic Aspects of Convalescence including Bone and Wound Healing, 10th Meeting, New York, June 15–16 (1945)
138 Marker, R E *J Am Chem Soc* **60**, 1725 (1938)
139 Marker, R E *ibid* **60**, 2442 (1938)
140 Marker, R E , and Kamm, O *ibid* **59**, 1373 (1937)
141 Marker, R E , Kamm, O , Crooks, H M , Jr , Oakwood, T S , Lawson, E J , and Wittle, E L *ibid* **59**, 2297 (1937)
142 Marker, R E , Kamm, O , Crooks, H M , Jr , Oakwood, T S , Wittle, E L , and Lawson, E J *ibid* **60**, 210 (1938)
143 Marker, R E , Kamm, O , and McGrew, R V *ibid* **59**, 616 (1937)
144 Marker, R E , Kamm, O , Oakwood, T S , Wittle, E L , and Lawson, E J *ibid* **60**, 1061 (1938)
145 Marker, R E , Kamm, O , Wittle, E L , Oakwood, T S , and Lawson, E J *ibid* **60**, 1067 (1938)
146 Marker, R E , and Lawson, F J *ibid* **60**, 2928 (1938)
147 Marker, R E , Lawson, E J , Rohrmann, E , and Wittle, E L *ibid* **60**, 1555 (1938)
148 Marker, R E , Lawson, E J , Wittle, E L , and Crooks, H M , Jr *ibid* **60**, 1559 (1938)
149 Marker, R E , and Rohrmann, E *ibid* **60**, 1565 (1938)
150 Marker, R E , and Rohrmann, E *ibid* **61**, 2537 (1939)
151 Marker, R E , and Rohrmann, E *ibid* **61**, 3022 (1939)
152 Marker, R E , and Rohrmann, E *ibid* **61**, 3476 (1939)
153 Marker, R E Rohrmann, E , and Wittle, E L *ibid* **60**, 1561 (1935)
154 Marker, R E , and Turner, D L *ibid* **62**, 2540 (1940)
155 Marker, R E , and Wittle E L *ibid* **61**, 855 (1939)
156 Marker, R E , Wittle, E L , and Lawson, E J *ibid* **60**, 2931 (1938)
157 Marrian, G F , and Butler, G C *Nature* **142**, 400 (1938)
158 Marrian, G F , and Butler, G C *Biochem J* **38**, 322 (1944)
159 Mason, H L *J Biol Chem* **124**, 475 (1938)
160 Mason, H L *Endocrinology* **25**, 405 (1939)
161 Mason, H L *J Biol Chem* **158**, 719 (1945)
162 Mason, H L , Hoehn, W M , McKenzie, B F , and Kendall, E C *ibid* **120**, 719 (1937)
163 Mason, H L , and Kepler, E J *ibid* **160**, 255 (1945)

164 Mason, H L., and Kepler, L J *ibid* **161**, 235 (1945)
165 Mason, H L, and Kepler, E J *ibid* **167**, 73 (1947)
166 Mason, H L, Myers, C S, and Kendall, E C *ibid* **114**, 613 (1936)
167 Mason, H L, Myers, C S, and Kendall, E C *ibid* **116**, 267 (1936)
168 Meyer, K *Helv Chim Acta* **29**, 718 (1946)
169 Meystre, C, Ehmann, L, Neher, R., and Miescher, K *ibid* **28**, 1252 (1945)
170 Meystre, C, Frey, H, Wettstein, A, and Miescher, K *ibid* **27**, 1815 (1944)
171 Miescher, K, Hunziker, F, and Wettstein, A *ibid* **23**, 400, 1357 (1940)
172 Miescher, K., and Wettstein, A. *ibid* **22**, 1262 (1939)
173 Miller, A M, and Dorfman, R I *Endocrinology* **37**, 217 (1945)
174 Miller, A M, Dorfman, R. I, and Sevringhaus, E L *ibid* **38**, 19 (1946)
175 McKenzie, B F, McGuckin, W F, and Kendall E C *J Biol Chem* **162**, 555 (1946)
176 Odell, A D, and Marrian, G F *ibid.* **125**, 333 (1938)
177 Olson, R E, Thayer, S A, and Kopp, L J *Endocrinology* **35**, 464 (1944)
178 Oppenauer, R *Z physiol Chem.* **270**, 97 (1941)
179 Ott, G H, and Reichstein, T *Helv Chim Acta* **26**, 1799 (1943)
180 Pearlman, W H, and Pincus, G *Federation Proc* **1**, 66 (1942)
181 Pearlman, W H, Pincus, G, and Werthessen, N T *J Biol Chem* **142**, 649 (1942)
182 Perla, D, and Marmorston Gottesman, J *Proc Soc Exptl Biol Med* **28**, 1024 (1931)
183 Petrow, V A, and Starling W W *J Chem Soc* **1946**, 749
184 Pfiffner, J J, and Vars, H M *J Biol Chem* **106**, 645 (1934)
185 Pfiffner, J J, Wintersteiner, O, and Vars, H M *ibid* **111**, 585 (1935)
186 Pincus, G Unpublished observations
187 Pincus, G, and Pearlman, W H *Vitamins and Hormones* **1**, 293 (1945)
188 Plattner, Pl A., and Heusser, H *Helv Chim Acta* **27**, 748 (1944)
189 Plattner, Pl A., Petrzilka, T, and Lang W *ibid* **27**, 573 (1944)
190 Plattner, Pl A, Ruzicka, L, Heusser, H, and Anglker, E *ibid* **30**, 395 (1947)
191 Plattner, Pl A, Ruzicka, L, Heusser, H., Pataki, J, and Meier, Kd *ibid* **29**, 942 (1946)
192 Plattner, Pl A, and Schreck, W *ibid* **24**, 472 (1941)
193 Prelog, V, and Tagmann, E *ibid* **27**, 1867 (1944)
194 Press, J, Grandjean, P, and Reichstein, T *ibid* **26**, 598 (1943)
195 Prins, D A Dissertation, Basel (1942)
196 Prins, D A, and Reichstein, T *Helv Chim Acta* **23**, 1490 (1940)
197 Reich, H *ibid* **28**, 892 (1945)
198 Reich, H, and Reichstein, T *ibid* **22**, 1124 (1939)
199 Reich, H, and Reichstein, T *ibid* **26**, 562 (1943)
200 Reich, H, and Reichstein, T *ibid* **26**, 2102 (1943)
201 Reichstein, T *ibid* **19**, 29 (1936)
202 Reichstein, T *ibid* **19**, 223 (1936)
203 Reichstein, T *ibid* **19**, 402 (1936)
204 Reichstein, T *ibid* **19**, 979 (1936)
205 Reichstein, T *ibid* **19**, 1107 (1936)
206 Reichstein, T *ibid* **20**, 953 (1937)
207 Reichstein, T *ibid* **20**, 978 (1937)
208 Reichstein, T, in Abderhalden, E Handbuch der biologischen Arbeitsmethoden, Berlin and Vienna, Abt V, Teil 3B, 1367 (1938)
209 Reichstein, T, and von Euw, J *Helv Chim Acta* **21**, 1197 (1938)

210 Reichstein, T , and Gatzi, K *ibid* **21**, 1185 (1938)
211 Reichstein, T , and Reich, H *Ann Rev Biochem* **15**, 155 (1946)
212 Reichstein, T , and Shoppee, C W *Vitamins and Hormones* **1**, 345 (1945)
213 Reinecke, R M , and Kendall, E C *Endocrinology* **31**, 573 (1942)
214 Rittenberg, D , and Bloch, K *J Biol Chem* **160**, 417 (1945)
215 Rittenberg, D , and Schoenheimer, R *ibid* **121**, 235 (1937)
216 Ruzicka, L , and Muller, P *Helv Chim Acta* **22**, 416 (1939)
217 Ruzicka, L , and Muhr, A C *ibid* **27**, 503 (1944)
218 Salmon, U J , Geist, S H , and Salmon, A A *Proc Soc Exptl Biol Med* **47**, 279 (1941)
219 Sarrett, L H *J Biol Chem* **162**, 601 (1946)
220 Schiller, S , and Dorfman, R I *Endocrinology* **33**, 402 (1943)
221 Schiller, S , Miller, A M , Dorfman, R I , Sevringhaus, E L , and McCullagh, E P *ibid* **37**, 322 (1945)
222 Schoenheimer, R , and Breusch, F *J Biol Chem* **103**, 439 (1933)
223 Schwenk, E , Riegel, B , Moffett, R B , and Stahl, E *J Am Chem Soc* **65**, 549 (1943)
224 Seebeck, E , and Reichstein, T *Helv Chim Acta* **26**, 536 (1943)
225 Segaloff, A , and Nelson, W O *Endocrinology* **31**, 592 (1942)
226 Selye, H *Science* **94**, 94 (1941)
227 Selye, H Encyclopedia of Endocrinology Franks, Montreal, 1943, Textbook of Endocrinology *Acta Endocrinologica*, Montreal, 1947
228 Shipley, R A , Horwitt B N , and Dorfman, R I *Am J Physiol* **139**, 742 (1943)
229 Shoppee C W *Helv Chim Acta* **23**, 740 (1940)
230 Shoppee, C W *ibid* **23**, 925 (1940)
231 Shoppee, C W *J Chem Soc* **1946**, 1139
232 Shoppee, C W , and Reichstein, T *Helv Chim Acta* **23**, 729 (1940)
233 Shoppee, C W , and Reichstein, T *ibid* **24**, 351 (1941)
234 Shoppee, C W , and Reichstein, T *ibid* **26**, 1316 (1943)
235 Simpson S L , and Joll, C A *Endocrinology* **22**, 595 (1938)
236 Smith, E R , Hughes, D , Marrian, G F , and Haslewood, G A D *Nature* **132**, 102 (1933)
237 Sonderhoff, R and Thomas, H *Ann* **530**, 195 (1937)
238 Sorkin, M , and Reichstein T *Helv Chim Acta* **27**, 1631 (1944)
239 Sorkin M , and Reichstein, T *ibid* **28**, 875 (1945)
240 Sorkin M , and Reichstein, T *ibid* **29**, 1218 (1946)
241 Spring, F S *Ann Rept Chem Soc* **40**, 147 (1943)
242 Steiger, M , and Reichstein, T *Helv Chim Acta* **20**, 817 (1937)
243 Steiger, M , and Reichstein, T *ibid* **20**, 1164 (1937)
244 Steiger, M , and Reichstein, T *ibid* **21**, 161 (1938)
245 Steiger, M , and Reichstein, T *ibid* **21**, 171 (1938)
246 Stevenson, J A F , Schenker, V , and Browne, J S L *Proc Assoc Internal Secretions* Chicago Meeting (1944)
247 Swingle, W W , and Pfiffner, J J *Science* **72**, 75 (1930)
248 Talbot, N B , Butler, A M , and Berman, R A *J Clin Investigation* **21**, 559 (1942)
249 Talbot N B , and Entingon, I V *J Biol Chem* **154**, 605 (1944)
250 Talbot, N B , Saltzman, A H , Wixom, R L , and Wolfe, J K *ibid* **160**, 535 (1945)

251 Thorn, G W, Engel, L L, and Lewis, R A *Science* **94**, 348 (1941)
252 Thorn, G W, Prunty, F T G, and Forsham, P H *Science* **105**, 528 (1947)
253 Venning, E H *J Biol Chem* **119**, 473 (1937), **126**, 595 (1938)
254 Venning, E H *Endocrinology* **39**, 203 (1946)
255 Venning, E H, and Browne, J S L *ibid* **21**, 711 (1937)
256 Venning, E H, Hoffman, M M, and Browne, J S L *ibid* **35**, 49 (1944)
257 Venning, E H, and Kazmin, V E *ibid* **39**, 131 (1946)
258 Venning, E H, Kazmin, V E, and Bell, J C *ibid* **38**, 79 (1946)
259 Venning, E H, Weil, P G, and Browne, J S L *J Biol Chem* **128**, cvii (1939)
260 Waterman, L, Danby, M, Gaarenstroom, J H, Spanhof, R W, and Uyldert, J E *Acta Brevia Neerland Physiol Pharmacol Microbiol* **9**, 75 (1939)
261 Weil, P G Quoted by Venning, Hoffman, and Browne (259)
262 Weil, P G, and Browne, J S L *Science* **90**, 445 (1939)
263 Westphal, U *Z physiol Chem* **273**, 13 (1942)
264 Wettstein, A *Helv Chim Acta* **23**, 388 (1940)
265 Wettstein, A *ibid* **23**, 388, 1371 (1940), **24**, 311 (1941)
266 Wettstein, A, and Hunziker, F *ibid* **23**, 764 (1940)
267. Wintersteiner, O, and Moore, M *J Am Chem Soc* **65**, 1503 (1943)
268 Wintersteiner, O, and Pfiffner, J J *J Biol Chem* **111**, 599 (1935)
269 Wintersteiner, O, and Pfiffner, J J *ibid* **116**, 291 (1936)
270 Wolfe, J K, Fieser, L F, and Friedgood, H B *J Am Chem Soc* **63**, 582 (1941)
271 Zwemer, R L, Lowenstein, B E, and Pines, K L *Endocrinology* **27**, 945 (1940)

Chapter XIV

Chemistry of Anterior Pituitary Hormones

By CHOH HAO LI AND HERBERT M EVANS

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I. Gonadotrophic Hormones

From the work of Smith (212), Zondek and Aschheim (236), and a number of later investigators (225), it was clear that pituitary extracts or implants produce two gonadal reactions (*a*) the stimulation of follicular growth in the ovaries and of spermatogenic activity in the testis and (*b*) the final ripening of the ovarian follicles together with the exhibition of estrus or heat, the rupture of the follicles and their transformation to corpora lutea, and in the testis the assumption of a functional role on the part of the Leydig cells, which ostensibly secrete testosterone, which in turn causes the development and assumption of function on the part of the secondary sex glands Whether or not these reactions are due to one or more hormones in the pituitary has been the subject of a number of investigations (215) Some investigators inferred from their physiological experiments that there was only one gonadotrophic hormone and that a difference in mode of administration or in dosage determined the type of reaction observed They would not feel that their contention is negated by the chemical fractionation of two substances in pure or almost pure form from pituitary tissue and by the experimental replacement of all gonadotrophic functions of the pituitary by the admin-

istration of these substances We will not further discuss this dispute to which more extended reference is made elsewhere but will content ourselves with an account of the chemical methods which have led to the separation of two pituitary gonadotrophic substances and of the chemical characterization of those substances

The first separation of pituitary gonadotrophic fractions into two components was obtained by Fevold Hisaw, and Leonard (69) in 1931 Subsequent work from other laboratories (11,103 227,228) confirmed the concept that two hormones are present in pituitary extracts—hormones which exhibit two kinds of gonadotrophic activity They have been designated the follicle stimulating hormone (FSH) or thylakentrin (37) and the interstitial cell stimulating hormone (ICSH) or metakentrin (137) The latter was first called the luteinizing hormone or LH

In 1940 two laboratories independently announced the isolation of ICSH in pure form from sheep (138) and swine glands (203) The preparations appear homogeneous in electrophoretic, ultracentrifuge, and solubility studies,[1] and it is particularly important that they are freed from contamination with the other gonadotrophic hormone, FSH On the other hand highly purified FSH preparations have been reported (80 89) though this hormone has not been isolated in pure state

Recently Evans Simpson, Lyons and Turpeinen (59) showed that lactogenic hormone can awaken or intensify corpus luteum function causing placentoma production in normal adrenalectomized, and hypophysectomized animals It should therefore be considered the third member of the pituitary gonadotrophic complex The chemistry of this hormone will be presented in another section

A Interstitial Cell Stimulating Hormone (ICSH or LH)

1 *Methods of Assay*

Many methods for the determination of the potency of ICSH have been proposed from different laboratories There is as yet no standard method to establish an international unit so that the potency of hormonal preparations from different laboratories can be compared In the following the commonly accepted methods are summarized

a Increase of Ovarian Weight in Normal Immature Rats This method (65) depends on the first-known characteristic of the hormone—that of producing copora lutea in the ovaries of immature female rats and of increasing the weight of the ovary when injected in combination with FSH Twenty one day old female rats of 35–40 g body weight are used Before the injection of ICSH, the ovarian weight of the

[1] For a discussion of criteria of purity in proteins see (183 202)

animal is caused to increase 100% over that of the control by the administration of FSH for four days Different dosages of ICSH are then injected subcutaneously twice daily over a period of five days simultaneously with the same amount of FSH The ovaries are weighed and examined on the sixth day A unit of ICSH is defined as the amount of hormone which produces an additional 100% increase in the ovarian weight together with the production of corpora lutea (67) The fact that the method is based on the action of two hormones makes it difficult to determine quantitatively the content of ICSH in crude or partially purified extracts, where an unknown amount of both hormones is already present However, the method may be useful in the standardization of the pure hormone

b Repair of Ovarian Interstitial Tissue in Hypophysectomized Rats As the name of the hormone indicated, ICSH is selectively able to repair the degenerated interstitial cells (deficiency cells) in the ovaries of hypophysectomized animals, *i e*, it causes the resumption of a normal nuclear picture, abolishing the agminated and wheel pattern of the nuclear chromatin The method as developed by Simpson *et al* (207) is based on this characteristic of the hormone The rats are used at a standard age (26–28 days) and postoperative period (6–8 days) The routine injection procedure is once daily, intraperitoneally for three days followed by autopsy 72 hours after the first injection The ovaries are sectioned for histological examination The amount of protein giving a minimal but definite effect on the "deficient' interstitial cells is called an ICSH unit It must be mentioned that if the subcutaneous route of injection is used, the test is one fifth as sensitive

c Increase of Seminal Vesicle Weight in Normal Immature Rats The known action of ICSH in male rats is also the stimulation of the interstitial cells, which in this case produce androgen, which in turn causes a weight increase of the secondary sex organs A test for ICSH potency in immature rats based on this principle has been proposed by Fevold (65,67) Male rats 22 days old are injected twice daily for four or five days, the animals are autopsied 24 hours after the last injection The seminal vesicles plus the coagulation glands are dissected and weighed A unit is defined as the amount of hormone which produces 100% increase in these combined weights as compared with the weight of controls Fevold found that the method could be employed to estimate the ICSH content in unfractionated extracts and believed that it gave consistent and reliable results Although the method appears convenient and simple, the sensitivity of the method is unfortunately greatly influenced by the strain of animals used In rats of the Long Evans strain, the seminal vesicles are hardly hypertrophied after the injection

of ICSH or unfractionated extract, the same materials, however, cause as high as 400% increase in the seminal vesicle weights in the Sprague Dawley strain of rats (67,76)

d Increase in Weight of the Ventral Lobe of the Prostate in Hypophysectomized Male Rats Greep *et al* (91) have pointed out the relatively greater reactivity of the ventral lobe of the prostate in the measurement of ICSH activity Hypophysectomized male rats (21 days old at operation, two days postoperative at the beginning of injections) are injected subcutaneously daily for four days with autopsy on the fifth day, 24 hours after the last injection Greep *et al* propose as standard dose (unit) the amount of hormone causing a 100% increase in weight of the ventral prostate as compared with untreated controls (92) The fact that the presence of FSH does not potentiate the ICSH activity makes the method particularly valuable We have confirmed the usefulness and reliability of the method and found further that the test becomes more sensitive when injections are made intraperitoneally instead of subcutaneously (207)

e Other Methods Witschi (234) suggests the use of the melanin reaction in the pectoral or abdominal feathers of African weaver finches as a measure of ICSH activity The weight increase of the testes of immature pigeons or of one-day-old chicks has also been proposed for the standardization of ICSH (208)

2 *Methods of Isolation*

The ICSH content of pituitary tissue from different animals has been studied by a number of investigators (65,229,234) We may summarize by stating that the ICSH content of pituitaries decreases in the following order sheep rabbit swine, rat, dog, horse, man, and beef It is obvious that the pituitary glands commonly used for the isolation of ICSH are those from sheep or pigs These glands as secured from slaughter houses may be either stored in a frozen state or desiccated with acetone [2] We have found that no loss of hormonal activity can be detected when glands are kept at −15°C for over a year

The solvents generally used for the extraction of the gonadotrophic principles from the pituitary are weak aqueous alkalis or dilute alcohol—saturated barium hydroxide (54), 0 02 *N* ammonium hydroxide (5), 2% pyridine (65), and 40% alcohol (228) Fevold (65) has found that pyridine is not only a good extractive but serves also as a preservative In a later report, Fevold *et al* (70) favor dilute ammonium hydroxide as the extracting agent

[2] Kupperman Elder, and Meyer (110) have investigated various methods of reserving pituitary glands

a Isolation of Sheep ICSH The method of Li, Simpson, and Evans (138,139,140) for the isolation of ICSH from sheep glands as a pure protein is as follows

(*1*) Three hundred grams acetone-desiccated whole sheep pituitary tissue is extracted with 4 l 40% alcohol over a period of two days with constant stirring and the residue is reextracted similarly using 2 l of the alcohol The supernatant liquids are combined and filtered through coarse folded filter paper The clear filtrate is brought to 80 or 85% alcohol by the addition of 95% alcohol and adjusted to pH 5 5 by the addition of glacial acetic acid The precipitate formed is removed by centrifuging and dried with absolute ethyl alcohol and ether The following steps are carried out at 5°C

(*2*) Fifty grams of this powder are extracted with 3 l distilled water The supernatant is mixed with an equal volume of acetone at about pH 4 5 The precipitate is next extracted with 1 l 1% sodium chloride solution

(*3*) The 1% saline extract is brought to 0 5 saturation with solid ammonium sulfate The precipitate is removed by centrifugation and the supernatant liquid is saved for purification of the FSH

(*4*) The 0 5 SAS[3] precipitate is dissolved in distilled water and brought to 0 2 saturation with the addition of SAS The supernatant liquid is next brought to 0 4 SAS

(*5*) The 0 4 SAS precipitate is dissolved in distilled water[4] and brought to 0 37 AS and SAS is added to the supernatant to 0 4 saturation

(*6*) The precipitate between 0 37 and 0 4 SAS is dissolved in distilled water and brought to 2 5% trichloroacetic acid by the addition of a 10% solution The precipitate formed is dissolved in a small volume of an aqueous alkaline solution and dialyzed The ICSH is obtained in dry form by lyophilization

The hormone thus obtained is free of other hormonal contaminants When 3 mg of the material is injected subcutaneously[5] in immature hypophysectomized female rats, no histologically detectable follicular stimulation is observed On the other hand, 0 005 to 0 01 mg of the same substance causes repair of the ovarian interstitial tissue in such rats on intraperitoneal injection In the same type of animal, 2 mg of the preparation is free of thyrotrophic hormone, 10 mg free of adrenocorticotrophic and of growth hormone In pigeons, 10 mg shows no crop stimulating activity

When the preparation is subjected to electrophoretic, ultracentrifuge, and solubility tests (140), it behaves as a homogeneous protein

Fevold *et al* (65,70) have also described a method for obtaining a highly purified sheep ICSH but their preparation contains two components in electrophoretic and ultracentrifugal analysis

[3] SAS will be used throughout this chapter as an abbreviation for saturated ammonium sulfate

[4] At this step an alternative procedure has been described for obtaining pure ICSH by precipitation at pH 4 0 to 4 1 in 0 33 SAS solution (140)

[5] See page 643 for testing FSH

b Isolation of Swine ICSH Chow, van Dyke, and co-workers (35,203) have described a method of obtaining ICSH from the glands of swine in pure form Their procedure is outlined below

(*1*) Four and one-half kg of ground fresh swine glands are extracted with 22 5 l 2% sodium chloride at 4°C The supernatant liquid is made acid with hydrochloric acid to pH 4 2 to 4 6

(*2*) To the supernatant, solid ammonium sulfate is added to saturation The precipitate formed is dissolved in water and dialyzed The dialyzed soluble solution is next adjusted to pH 5 1 to 5 5

(*3*) The supernatant fluid is made to 0 33 saturation with ammonium sulfate The 0 33-SAS-soluble fraction is brought to 0 9 saturation

(*4*) The precipitate is dissolved in water and dialyzed ammonium sulfate is added to the dialyzed solution until 0 33 saturation After centrifuging off the small amount of precipitate, the supernatant is adjusted to pH 7 3

(*5*) The precipitate formed is dissolved in water and step 4 is repeated at least seven times The final pH 7 3 precipitate at 0 33 SAS is the pure hormone

The preparation isolated shows homogeneity in electrophoretic, ultra centrifugal, and solubility tests The preparation was examined in immature hypophysectomized female rats for the detection of FSH adrenocorticotrophic, and thyrotrophic contaminants and these were found to be absent in the large dose employed (0 34 mg daily for ten days) No data however have been furnished as to the absence of the growth and lactogenic hormones

3 *Comparison of Swine and Sheep ICSH*

a Physicochemical Properties From osmotic pressure measurements, a molecular weight of 40,000 is obtained for sheep ICSH whereas the swine hormone has a value of 100 000 as calculated from ultracentrifugal data The sedimentation constants, S_{20}, of sheep and swine ICSH are $3\,6 \times 10^{-13}$ and $5\,4 \times 10^{-13}$, respectively The latter value, if corrected for the viscosity and density of the solvent, becomes $6\,8 \times 10^{-13}$

Electrophoretic experiments also reveal dissimilarity in the protein hormones isolated from these two different species At pH 7 85 and ionic strength 0 05 buffer, swine ICSH migrates with a rate of $0\,52 \times 10^{-5}$ cm ^{2}/sec /v , while the sheep hormone has an electrophoretic mobility of $6\,36 \times 10^{-5}$ in a buffer of pH 7 53 and ionic strength 0 05 Sheep ICSH possesses an isoelectric point at pH 4 6 and swine at pH 7 45

This difference in the electrical properties of swine and sheep ICSH has been further verified by Chow *et al* (35) They have compared the electrophoretic behavior of the swine hormone with that of a highly purified sheep ICSH prepared by Jensen Although Jensen's preparation contained two components they were able to show that the biological activity is associated with the main component and that the isoelectric point of the main component may be estimated at about pH 4 8 to 5 0

Both sheep and swine ICSH contain carbohydrate but the content differs significantly The sheep hormone has 4 5% mannose and 5 8% hexosamine, while swine has 2 8% mannose and 2 2% hexosamine (93,138) The tryptophan content of the two hormones has also been found to be different By the glyoxylic acid method, swine ICSH is shown to contain 1 0% tryptophan, the hormone isolated from sheep glands on the other hand contains 3 8% tryptophan Table I gives a summary of the physicochemical data for ICSH as isolated from sheep and swine pituitary glands, respectively

TABLE I

PHYSICOCHEMICAL CHARACTERISTICS OF SHEEP AND SWINE ICSH*

Determinations	Sheep	Swine
C, %		49 37
H, %		6 83
N, %	14 20	14 93
Molecular weight	40 000	100 000
Isoelectric point, pH	4 6	7 45
Sedimentation constant, $S \times 10^{13}$	3 6	5 4
Tyrosine, %	4 5	
Tryptophan, %	1 0	3 8
Mannose, %	4 5	2 8
Hexosamine, %	5 8	2 2

* See (93 138 140 203)

b Immunological Specificity Perhaps the most sensitive test for species differences in proteins is obtained from immunological reactions Chou (31) has made such studies with swine and sheep ICSH When the pure swine ICSH is injected into rabbits, the production of specific antibodies can be demonstrated both by the precipitin and complement fixation reactions The antiserum[6] thus obtained does not react with pure sheep ICSH or extracts prepared from sheep glands It would therefore appear that the interstitial cell stimulating hormones isolated from swine and sheep glands are chemically different entities

c Biological Potency of Swine and Sheep ICSH Since the sheep and swine hormones have been shown to be different substances it would not be unexpected that they should differ in biological potency Some differences have been reported Greep *et al* (92) have found that the

[6] Chow has further demonstrated that the swine ICSH is not only "lobe specific" but also 'hormone specific' The immulogical study may therefore serve as a highly sensitive and specific test for hormonal contaminants in any supposedly pure hormone preparation

sheep ICSH is far more active in the repair of the ovarian interstitial cells in hypophysectomized rats and in causing ovulation in rabbits than is the swine hormone On the other hand, the two hormones are equally effective in stimulating the anterior prostate of hypophysectomized rats Table II presents a comparison of the ICSH potency of these two hormones as determined by different tests

TABLE II

BIOLOGICAL POTENCY OF PURE SWINE AND SHEEP ICSH[a]

Test	Sheep	Swine
Total dose necessary to increase weight of ventral prostate from 6 39 to 9 45 mg in hypophysectomized rats	0 0134 mg	0 0134 mg
Total dose necessary to repair ovarian interstitial cell in hypophysectomized rats	0 005 to 0 010	>0 10
Total dose necessary per kg body weight to produce positive response in ovulation of all of 8 rabbits	0 007	>0 040

[a] See (92)

4 *Effect of Various Agents on ICSH Activity Chemical Differences between FSH and ICSH*

Since the original observation of Fevold *et al* (69) that FSH is more soluble in aqueous salt solution than ICSH, a considerable number of studies has been made as to chemical differences in the two hormones on treatment with various reagents Although almost all experiments have employed impure preparations, the results obtained are of value in our understanding of the intrinsic nature of the hormones

a Effect of Ketene Ketene is considered to be a mild and specific acetylating agent for aqueous protein solutions Three groups in proteins are known to react with this agent *viz* the amino, phenolic hydroxyl, and sulfhydryl groups Both purified FSH and ICSH fractions have been subjected to ketenization at room temperature (137) After five minutes treatment, the interstitial cell stimulating activity is greatly reduced while the follicle stimulating action is apparently unchanged Upon longer treatment (thirty minutes), both hormonal activities are almost completely destroyed The inactivation of ICSH by ketene was later confirmed with a pure preparation (138), based on results obtained by other investigators, it is assumed that the free amino groups are essential for the biological activity of ICSH Though it is admitted that such assumptions are invalid without complete chemical analysis of the active groups in acetylated samples, nevertheless the results obtained suggest

distinct chemical differences between ICSH and FSH in the manner of inactivation by ketene

b Effect of Cysteine It is generally agreed that cysteine reduces —S—S— cross links in proteins at an alkaline pH, i e , thiol groups result and a corresponding amount of cysteine is oxidized to cystine Fraenkel-Conrat *et al* (79) have used this reducing agent to investigate the essentiality of disulfide (—S—S—) groups in gonadotrophic hormones When FSH and ICSH preparations are allowed to react with cysteine (forty times the amount of protein) at pH 7 7 for two days, their biological activities are greatly decreased In a later report (81), no loss of gonadotrophic potency was noted under conditions of cysteine treatment milder than those employed for the inactivation of insulin It appears that the disulfide groups in these gonadotrophic substances are not so readily reduced as is the case with other proteins They conclude that the integrity of some disulfide bonds which are not easily reduced is essential for hormonal activity The inactivation of FSH by cysteine has been confirmed by McShan and Meyer (163) The results of Bischoff's experiments (18) are also in complete agreement with those reported by Fraenkel-Conrat *et al*

c Effect of Protein Precipitants It was noted in the course of the purification of ICSH that FSH was soluble in 2 5% trichloroacetic acid, whereas ICSH was completely precipitated in the same solution (138) By using other protein precipitants, Fevold (64) has also observed chemical differences in the two hormones, FSH was found to be inactivated by picrolonic, picric, and flavianic acids, whereas ICSH retained its activity on treatment with these reagents While we must note that these results were not confirmed by Jensen *et al* (104), it is important to remember that the disagreement could be due to differences in the assay methods employed, the strain of animals, or in the purity of the hormones In view of our experiments with trichloroacetic acid we view it as likely that the two hormones can be observed to differ in their reactions with various reagents

There is another protein precipitant, namely tannic acid, which has been used for some time in augmenting FSH or ICSH potency (68,76) The augmenting effect of tannic acid and other agents on the two hormones is discussed elsewhere in this volume

d Effect of Enzymes In 1929 Reiss and Haurowitz (189) discovered that crude trypsin destroyed gonadotrophic activity in a pituitary extract Later, Bates, Riddle, *et al* (11,194) found the destructive action of a purified trypsin preparation on FSH However, McShan and Meyer (161) could not confirm these results, they stated that commercial trypsin destroyed almost all ICSH (88), but not FSH, activity The

results of Chen and van Dyke (30) and Greep (88) agreed with those of McShan and Meyer. On the other hand, Abramowitz and Hisaw (1) claimed that neither crystalline trypsin nor chymotrypsin inactivates ICSH more rapidly than FSH. It may be well to point out that all these results were obtained from assays in normal animals and furthermore that the extent of enzymic digestion in no case was determined.

TABLE III

EFFECT OF SOME PROTEOLYTIC ENZYMES ON GONADOTROPHIC POTENCY OF SWINE PITUITARY EXTRACT[a]

Enzyme	Protein digested, %	FSH	ICSH
Crystalline carboxypeptidase	5 (5 hr.)	Unaffected	Unaffected
	5–12 (30–41 hr.)	Unaffected or reduced	Reduced
Crystalline chymotrypsin	18–35	Reduced	Absent
	68–80	Absent	Absent
Crystalline trypsin	12–48	Reduced	Absent
	61–75	Usually absent	Absent
Merck's trypsin	10	Unaffected	Unaffected
	35–46	Unaffected or reduced	Reduced
	61–75	Unaffected or reduced	Absent
Papain	6–31	Unaffected	Unaffected
	60–65	Reduced	Reduced
Crystalline pepsin	10–34	Unaffected or reduced	Reduced?
	58–80	Absent	Reduced?

[a] Taken from Chow, Greep, and van Dyke (34).

The controversies concerning the trypsin experiments finally become clear. Chow, Greep, and van Dyke (34) have reinvestigated the effect of crystalline trypsin and commercial trypsin (Merck) on the gonadotrophic activity of pituitary (swine) extract in a most careful and thorough manner The rate of hydrolysis of the extract was estimated by determining the decrease of protein precipitable by trichloroacetic acid, and the destruction of gonadotrophic potency was followed by assays in immature hypophysectomized male or female rats. Using crystalline trypsin, Chow *et al* found that with over 60% digestion all gonadotrophic activity is lost while when the amount of digestion is between 12–48%, FSH appears to be more resistant than ICSH. If commercial trypsin is used, the disappearance of 10% of the protein is not followed by any destruction of FSH or ICSH potency, but, when higher percentages of protein are digested, ICSH appears to be selectively destroyed. The interesting results with crude trypsin cannot be explained by the presence of crystalline trypsin. Chow *et al.* conclude that "the proteolytic

activity of Merck's trypsin depends to a major extent on the presence of enzyme(s) other than trypsin or chymotrypsin" Chow, Greep, and Van Dyke (34) have also studied the effect of crystalline pepsin on FSH and ICSH potency Their results are summarized in Table III

Another difference in the reactivity of the two gonadotrophic hormones toward enzymic digestion is shown by the experiments of McShan and Meyer (161,162), who found that ptyalin (saliva) abolished the follicle-stimulating activity, the luteinizing activity being relatively resistant

B Follicle-Stimulating Hormone (FSH)

1 *Methods of Assay*

a Normal Immature Female Rats Since FSH stimulates follicular growth, it is obvious that one may use increase in ovarian weights as contrasted with those of controls to determine rat units (63,70) Fevold and co-workers have employed this method routinely for the quantitative estimation of FSH potency It must be emphasized that FSH is potentiated by the presence of ICSH and that, if the animal's own pituitary is present or if FSH fractions are not free from ICSH, the assay results obtained in normal immature animals do not represent absolute units of FSH

Another procedure, based on the fact that FSH exerts an augmentation effect on the activity of the gonadotrophic principle in pregnant women's urine (CG, chorionic gonadotropin) in immature female rats, has been reported by Evans *et al* (61) A unit is defined as the minimal amount of material which, given subcutaneously to 24 26-day old female rats in combination with a standard amount of CG, augments the effect of the latter by 100% The difficulties of this method are the presence of ICSH in FSH preparations will render the test less sensitive and it gives no information regarding ICSH contamination It is therefore advisable to assay FSH preparations in hypophysectomized animals for quantitative data

b Hypophysectomized Rats (*1*) *Female* In hypophysectomized animals FSH causes the enlargement of the ovarian follicles while leaving the interstitial tissue in the deficient condition if, of course, the preparation is not contaminated with the luteinizing factor In the assay of FSH in these animals, either increase of ovarian weights or histological examination for beginning follicular development may be taken for measurement of the hormone One rat unit represents the minimal total amount which, injected subcutaneously once daily over a period of three days into hypophysectomized rat (26–28 days old at operation, six- to eight-

day postoperative interval before injection), causes the occurrence of healthy (nonatretic) follicles with small antra, as evidenced 72 hours after beginning the injection (61) For routine laboratory assay, three animals per group can furnish a reliable answer to hormonal potency The method is very sensitive and has the further advantage that the presence of ICSH can be observed simultaneously if there is repair of the interstitial tissue

(*2*) *Male* The follicle stimulating hormone is known to stimulate the epithelium of the seminiferous tubules As shown by Greep *et al* (90,92), FSH causes an increment in testis weights proportional to the dose injected without any stimulation of the secondary sex organs in hypophysectomized male rats The animals employed were hypophysectomized at 21 days of age (35–47 g body weight), after two days postoperative injections began once daily for four days and the animals were autopsied on the day following the last injection If ICSH is a contaminant in the follicle stimulating preparation, the test becomes unreliable, for ICSH alone is able to increase the weight of the testes (209)

Other methods have been used by investigators for the assay of FSH such as the increase of the uterine weights (115), the production of oestrous vaginal smears (234), etc

2 *Methods of Purification*

Sheep and swine pituitaries are rich in follicle-stimulating substance and they are therefore commonly used to obtain a potent FSH preparation The extraction of pituitary tissue (fresh or acetone-dried material) is made either with saline or alcoholic solutions like those employed for the isolation of ICSH Chemically the follicle-stimulating hormone is in one respect unique in that it is the only known anterior hypophyseal hormone soluble in half saturated ammonium sulfate Highly purified FSH possesses a high carbohydrate content In addition, as has been discussed, the hormone is resistant to tryptic digestion when a commercial enzyme preparation is employed The follicle stimulating hormone has not been isolated in pure form There are methods which enable one to obtain a so-called "biologically pure" preparation, *i e*, a preparation free from other active contaminants Subjoined, we give a few methods which appear to be satisfactory in preparing potent follicle-stimulating preparations

a Procedure of Fevold et al (70) One kg of frozen sheep glands are finely ground and extracted with 2 l of dilute aqueous ammonium hydroxide at pH 8 0 The supernatant liquids are brought to 0 25 *M*

ammonium sulfate and to pH 5 4 After the removal of the precipitate formed by centrifugation, the supernatant is adjusted to pH 7 0 and fractionated with ammonium sulfate The fraction soluble at 2 4 *M* but precipitated at 2 7 *M* ammonium sulfate is the follicle stimulating substance As assayed by increase in the ovarian weights of immature female rats, the product obtained contains 20 R U /mg No experimental data were given by Fevold *et al* as to possible contamination with other active components

In an earlier report (65), Fevold used 2% pyridine as the extractant The gonadotrophic substances are adsorbed by benzoic acid, the ICSH fraction is next removed by precipitation at pH 4 2 in 0 2 SAS the soluble material containing the FSH Inert substances in the FSH preparation are further removed by basic lead acetate FSH preparations thus obtained have a potency of 50 and 75 R U /mg

b Procedure of Fraenkel Conrat et al (80) From the fraction soluble in 0 5 SAS as described in step *3* for the isolation of sheep ICSH (see page 637), the supernatants are brought to 0 67 saturation with ammonium sulfate The precipitate obtained between 0 5 and 0 67 SAS contains the follicle-stimulating substance It can be further purified in the following ways precipitation at pH 4 8 in 35% acetone, precipitation in 48% alcohol in the presence of a few drops of saturated sodium chloride solution, and removal of contaminating proteins at pH 4 1 in saturated sodium chloride solution

The product obtained causes beginning ovarian follicular development at a total dose of 0 004 mg when injected subcutaneously in hypophysectomized rats Higher doses of the preparation (0 04 to 0 06 mg) show indications of ICSH contamination, i e luteinization of the follicular walls and repair of the interstitial tissue In addition, oestrous uteri are caused by 0 016 mg of the hormone

c Procedure of Greep et al (89) Greep, van Dyke, and Chow (89) have described a method for obtaining a so-called "biologically pure" follicle stimulating hormone from swine pituitary glands The method, which is based on the fact that FSH is soluble in a pH 4 4 acetate buffer containing 20 5% sodium sulfate whereas ICSH is insoluble in this solvent, is outlined as follows

(*1*) Fresh hog pituitaries are extracted with 2% sodium chloride at pH 4 2 The supernatant is saturated with ammonium sulfate The precipitate is dialyzed and the clear dialyzed solution is adjusted to pH 5 1 (*2*) The supernatant is brought to 50% saturation with ammonium sulfate at pH 4 2 The supernatant is further brought to 0 9 SAS (*3*) The precipitate is dialyzed until saltfree One volume of 1 *M* acetate buffer of pH 4 41 and 2 volumes of 41% sodium sulfate are added The precipitate contains no FSH and is used for the isolation of ICSH (*4*) The supernatant is made to 40% ammonium sulfate by adding 40 g ammonium sulfate/

100 ml (*5*) The precipitate is dialyzed until free of salt Steps *3* and *4* are repeated until no turbidity is observed in step *3*

The final precipitate (92) does not cause enlargement of the ventral prostate or stimulation of the ovarian interstitial cells in hypophysectomized rats when injected at a total dose of 2 77 mg protein (assuming the preparation contains 13% nitrogen), but 0 0154 mg produces an increase of 50% in ovarian weights over those of untreated controls The preparation did not give uterine stimulation at the highest dose tested (a total dose of 1 92 mg in ten days) It is apparent that the FSH prepared by Greep *et al* was free from ICSH contamination Unfortunately they did not give data to show that the preparation contained no adrenocorticotrophic, thyrotrophic, lactogenic, or growth activity so that its biological "purity" cannot be regarded as established

In a later communication, Chow (33) reported that ultracentrifugal and electrophoretic experiments indicated definitely the heterogeneity of their FSH preparation However, Greep *et al* (89) stated earlier that solubility studies in one solvent show "no evidence of contaminating proteins if the concentration of the solid phase is five times that saturating the solution though an increase in the amount of protein N is observed if the solid phase is hundred times that necessary for saturation '

d Procedure of McShan and Meyer (163) As already mentioned commercial trypsin preparations destroy only ICSH activity in gonadotrophic extracts and FSH is apparently resistant to the enzyme digestion McShan and Meyer utilize this fact and develop a method to prepare a biologically pure follicle stimulating substance Their method may be described in the following steps

(*1*) Acetone-dried sheep pituitary powder is extracted with water The gonadotrophic activity is precipitated from the supernatant by the addition of four volumes of acetone (*2*) The precipitate is further extracted with water the supernatant is digested at 38°C for 30 hours at pH 8 with 40 mg trypsin (Fairchild)/g of original pituitary powder (*3*) Insoluble material formed during digestion is centrifuged off the supernatant is placed in a 75° water bath for twenty minutes (*4*) The digest is dialyzed against 0 1 *M* acetate buffer of pH 4 0 The precipitate formed is discarded (*5*) The supernatant is mixed with four volumes of 95% alcohol the fine white precipitate is dried with alcohol and acetone

The final product is found to cause only follicular development in normal and hypophysectomized immature female rats in most cases The preparation is shown to be free from lactogenic and thyrotrophic activities as assayed in pigeons and chicks No physicochemical purity is given It is probable that the preparation contains a high percentage of inactive contaminants as judged by its ability to increase the ovarian weights of normal immature rats when compared with the preparation

obtained by Fraenkel-Conrat *et al.* (80). The preparation has been shown to cause local reactions at the site of injection when used in human subjects but, in a later report (164), McShan and Meyer introduce a procedure to remove the toxic substance.

TABLE IV

BIOLOGICAL POTENCY OF PURIFIED FSH PREPARED BY DIFFERENT LABORATORIES

Method of preparation	Normal immature rats				Hypophysectomized rats			
	Total dose, mg	Days of injection	No of rats	Ovarian weight, mg	Total dose, mg	Days of injection	No of rats	Ovarian weight, mg
Greep, van Dyke, and Chow (92)					0		28	8 63
					0 0625	4 (?)	4	12 47
					0 625	4	5	16 28
					1 562	10	6	15 10
					0	10	6	5 70
Fevold (66)	0			10	0			6
	0 02	4		18	0 02	4		10
	0 04	4		24	0 04	4		16
	0 08	4		32	0 08	4		24
	0 16	4		35	0 20	4		31
	0 50	4		44	0 50	4		35
McShan and Meyer (164)	5 8	4 5	6	85 0	31 0	10	5	263
	5 9	4 5	7	41 0	28 9	10	4	183
	6 6	4 5	3	110 0				
Fraenkel-Conrat, Simpson, and Evans (80,82)	0 010	3	3	26	0		100	11
	0 015	3	9	36	0 003	3	9	14
	0 025	3	12	38	0 015	3	8	30
	0 050	3	9	46	0 018	3	8	39
	0.10	3	3	54				

Table IV summarizes the biological potency of FSH fractions prepared by different laboratories. It is clear that the swine FSH prepared by Greep *et al* has a much lower follicle-stimulating potency than sheep hormone as reported by Fevold and Fraenkel-Conrat *et al.*, but the sheep FSH gives an ICSH reaction at very low doses while the swine FSH is apparently free from ICSH action. Some investigators attribute these differences to species characteristics (82,164) while others (33) feel that they are due to contamination of sheep FSH by ICSH. The final answer to the problem must await the isolation of FSH in pure form.

3 *Physicochemical Properties*

Gurin (93) reported that swine FSH obtained by the method of Greep, van Dyke, and Chow contains 4 5% mannose and 4 4% hexoseamine Evans *et al* (53) found that the potent FSH from sheep glands is rich in carbohydrate and glucoseamine McShan and Meyer (162 163) stated that their FSH preparations contain about 20% glucose In addition, FSH activity is destroyed by certain amylase preparations (45) It is probable that pure FSH is a glycoprotein It is of interest to note that all gonadotrophic hormones including those of nonpituitary origin seem to contain carbohydrate (93)

The preparation as described by Fraenkel-Conrat *et al* (80) contains 13 1% nitrogen It is generally agreed that FSH proteins are very soluble in water, in the absence of electrolytes, they are soluble in 50% acetone 70% alcohol (80), and 50% dioxane (63) The follicle stimulating potency is comparatively stable, in solutions of pH 7 to 8 the activity is retained at 75°C for thirty minutes (163), but it is destroyed at 60°C for fifteen minutes in 50% alcoholic solution (63) Chow (33) has estimated the isoelectric point of his FSH preparation from swine glands to be about 4 8

The effects of enzymes and of other chemical agents on the follicle-stimulating activity of pituitary extracts have already been discussed in this chapter

II. Lactogenic Hormone (Prolactin)

The first indication of the existence of a lactogenic substance in extracts of the anterior pituitary came from the experiments of Stricker and Grueter (216), who found that extracts initiate lactation in ovariectomized pseudopregnant rabbits The conclusion was later confirmed by Corner (44) and others (155,174,191) In 1932, Riddle *et al* (192,193) discovered the stimulating action of the lactogenic principle on the crop sac of the pigeon and suggested the name prolactin Two other names have been proposed galactin (86) and mammotropin (151)

A Methods of Assay

In estimating lactogenic potency two groups of methods have been employed one depends on the crop sac reaction in the pigeon and the other on the response of the mammary gland of the "conditioned" rabbit or guinea pig The latter methods (86,152,154,173), are less quantitative l are laborious, they will not be discussed here

1 *Crop Sac Weight Method*

This is the original method proposed by Riddle, Bates, and Dykshorn (193) They found that the combined weights of the two excised crop sacs are proportional to the amount of lactogenic principle injected Pigeons (six to ten weeks after hatching) are injected intramuscularly once daily for four days and autopsied about 96 hours after the first injection Under these conditions Riddle *et al* found that the crop weight is a linear function of the logarithm of the dosage The pigeons used must have approximately the same body weight because the crop sac weight depends on the size of the bird (197) If the crop weight is calculated in terms of a unit of body weight, *i e*, relative crop weight, a more consistent response–dosage relationship is obtained According to Folley *et al* (74) the accuracy of the weight method depends on three factors "(1) the standard deviation of a single observation, (2) the number of birds used in each group and (3) the slope of the dose–response curve"

Bates and Riddle (10) have found a seasonal variation in the response of crop sacs, maximum response occurs in winter and summer It is therefore essential to keep a standard preparation at hand and always compare the potency of an unknown with that of the standard To obtain uniform results, the birds must be of the same strain and race Bates *et al* (12) reported that the crop sac response changes with racial and strain differences Folley, Dyer, and Coward (74) investigated the effect of light and temperature on the crop sac response of pigeons, they found that light exerts no influence on the response and that a maximum response is achieved at an equable temperature in the region of 15°C The effectiveness of different routes of injections has been studied by Bates and Riddle (8) Subcutaneous injections are most effective, intraperitoneal ones least effective

2 *Minimum Stimulation Method*

In a footnote in their paper, Lyons and Catchpole (155) stated that "one need not depend upon a weight increase in the crop gland over and above the control to determine a positive reaction, since beginning growth changes may be seen in crops that weigh less than the average normal, as early as 48 hours after the injection of potent hormone" They suggest a qualitative test for lactogenic activity by merely holding the crop gland to the light to examine beginning recognizable stimulation McShan and Turner (165) proposed a quantitative assay method based on the suggestions of Lyons and Catchpole and defined a pigeon unit as "the total amount of hormone injected during a period of 4 days which cause a

minimal but definite proliferation of the crop glands of 50 ± 11 per cent in common pigeons weighing 300 ± 40 gm " In our laboratory, we have employed this method routinely to estimate lactogenic potency and have found that, even using only three birds per group, satisfactory results can be obtained Silver King pigeons, four to five weeks from hatching and weighing 400 to 550 g , are injected subcutaneously once daily for four days with 0 5 ml of the hormonal solution, 24 hours after the last injection, the crop is dissected and examined against the light for a positive reaction If two out of three birds give a positive response, the amount of hormone used is considered to be one unit

3 *Local Intradermal Method or Micro Method*

For the estimation of a minute quantity of lactogenic hormone, *e g* , that in urine, Lyons and Page (156) introduced a highly sensitive test by intracutaneous injection in the neck skin directly over the pigeon's crop sac According to Lyons (152), the hormone solution (0 1 ml) is injected intradermally within the skin covering the crop sacs, daily for four days with a 27 gage hypodermic needle The birds are sacrificed on the fifth day, the sacs are dissected off and held slightly stretched against the light when a positive response can be seen with the naked eye The injections are generally carried out over one crop sac and the sac on the opposite side may serve as a control or be injected with a different dose level The volume of injection fluid has some influence on the sensitivity of the method (9) It is therefore essential for quantitative results to utilize a constant volume It has been shown that the method will detect 1/10 000 of a unit obtained from the minimum stimulation test McQueen Williams (159) and others (185,186) have applied this method to detect the presence of lactogenic hormone in a single rat pituitary implanted directly over the crop sac

In 1938 arrangements were made for the establishment of an international unit (I U) of the lactogenic hormone during the Conference on the Standardization of Hormones (25) In the following year the international standard preparation of the hormone was issued, the international unit is defined as "the specific activity contained in 0 10 mg of the standard preparation" (26) It is therefore possible to state the potency of any lactogenic preparation in terms of the international unit Lyons (154) has made careful studies on the potency of the international standard material and found that it is about one half as potent as the pure lactogenic hormone in the guinea pig assay and about one third as potent by the crop weight response method

B Methods of Isolation

The content of lactogenic hormone in pituitary glands of different species has been reported by a number of investigators Bates and Riddle (7) have found that ox and sheep glands contain much higher concentrations of the hormone than do glands obtained from swine A later report by Chance *et al* (29) showed that the hormone content[7] is progressively smaller in the following order sheep, ox man, swine, and horse The content in horse glands amounts to only 4% of the amount found in sheep or ox Therefore the starting material for the isolation of the lactogenic hormone is usually either ox or sheep glands

The solvents which have been used by different investigators for the extraction of the lactogenic substance from pituitary tissue, are aqueous acid solutions of pH 2 (191), aqueous alkali solutions of pH 9 (86 192,193), 66% acetone in acid at pH 2 (155), 60–70% alcohol at pH 9–10 (7), and aqueous saline solution (22) Bergman and Turner (16) have made a comparison of these methods of extraction and conclude that the method of Bates and Riddle (7) is superior both for total yield and potency/mg extracted substance But the acid–acetone extraction of Lyons (152,153) has constituted the initial step in the isolation of the hormone in pure form A prime advantage of Lyon's extract is that it contains mainly only the lactogenic and adrenocorticotrophic hormones the other hormones being either not extractable with the solvent or destroyed by the acidic acetone

A highly purified and potent lactogenic hormone preparation was first described by Lyons (152,153) in 1937 In the same year, White *et al* (233) announced the preparation of a crystalline protein possessing crop stimulating activity, in the preliminary note, there was, however, no data concerning the biological and chemical purity of the crystalline preparation It was not until 1942 that White, Bonsnes, and Long (232) published a satisfactory identification of the crystalline protein with the hormone In the meantime (1940–1941) Li, Lyons, and Evans (129,130,132,133) showed that their lactogenic hormone preparation behaved like a pure protein as judged by electrophoretic and solubility studies

The original method of Lyons (153) for the isolation of lactogenic hormone in pure form as slightly modified by Li *et al* (129,130,132,133) may be described in the following steps

[7] For the content of lactogenic hormone in pituitaries of ox, mice, rats guinea pigs rabbits, and cats, see Reece and Turner (187)

(*1*) One kg of ground sheep pituitaries is extracted with 4 l acetone to which has been added 100 ml 12 *M* hydrochloric acid The extracted material is next precipitated out by the addition of 5 l acetone (*2*) Dissolve the precipitate in 200 ml 50% acetone and reprecipitate by adding 800 ml acetone The precipitate formed is dissolved in 300 ml of approximately 10% aqueous ammonium hydroxide (*3*) The solution is allowed to stand at room temperature for three hours Two volumes of acetone are added and if a precipitate forms this is removed by centrifugation (*4*) The supernatant is mixed with one volume of acetone and 10 ml 12 *N* hydrochloric acid The precipitate is dissolved in 200 ml water with the aid of 1 *M* sodium hydroxide to obtain a clear solution (*5*) The solution is adjusted to pH 6 5 with 1 *M* hydrochloric acid and the precipitate removed by centrifugation (*6*) The supernatant is brought to pH 5 5 and kept at −15°C for a few hours After thawing the solution is centrifuged (*7*) The precipitate is dissolved in slightly alkaline solution and steps *5* and *6* are repeated until the final pH 5 5 precipitate behaves as a single substance in electrophoretic experiments

The hormone thus isolated contains about 30 I U /mg as assayed by the minimum stimulation method in pigeons The preparation is free from other active components Electrophoretic studies (129,130,132) in buffers of pH from 2 to 8 show that the preparation migrates in the electrical field as a single protein Evidence for the purity of the preparation is also furnished by solubility experiments in three different solvents

The pure hormone can also be obtained by an alternate method as described by Li, Simpson, and Evans (141) or by the method of White *et al* (232)

C Physicochemical Properties

1 *Isoelectric Point*

From the pH at which the hormone becomes least soluble, it has been speculated that prolactin must possess an isoelectric point at approximately pH 5 5 The exact isoelectric point of the hormone as estimated by the moving boundary method in electrophoresis (129) is found to be pH 5 73 in buffer solutions of ionic strength 0 055 White *et al* (230 232) reported a value of pH 5 65 using the micro electrophoresis technique of Abramson (2) in buffers of ionic strength 0 10 The small difference between these two values is most probably due to the different ionic strengths employed and they should be considered to be in satisfactory agreement

2 *Molecular Weight*

The report of Lyons and Page (156) and others (49,99,113) on the detection of prolactin in urine would lead one to expect that the molecular weight of the hormone is comparatively low The first molecular weight data were obtained from osmotic pressure measurements (132) and indicated that the hormone has indeed a low molecular weight, *i e*, 26 500 From analytical data, the molecular weight may also be esti-

mated to be about 25,000 Diffusion and viscosity data suggest a value of 22,000 (see below)

The sedimentation constant of prolactin as determined in the analytical air-driven ultracentrifuge has been reported by White, Bonsnes, and Long (232) to be 2.8×10^{-13} cm /sec /dyne Preliminary data on the sedimentation and diffusion constants as secured in the laboratory of J W Williams were also reported by them to be $S_{20} = 2.65 \times 10^{-13}$, $D_{20} = 7.5 \times 10^{-7}$, from these values, a molecular weight of 32,000 for prolactin was computed Since no complete data were given the value 32,000 can only be assumed *tentatively* as the molecular weight of prolactin when determined by ultracentrifuge At any rate the difference in these two values (26,500 and 32 000) is hardly surprising for many experiments (179) have shown that molecular weight determinations as obtained by osmotic pressure or by ultracentrifugation methods need not be in close agreement The comment made by White (230) that the value 26,500 is "considerably too low" would appear to be gratuitous

3 *Diffusion and Viscosity*

The viscosity of prolactin solutions (117) has been determined in an Ostwald viscometer A straight line relationship exists between the viscosity and the protein concentration up to 1 0% solution From the slope of such a straight line, the hormone molecule is demonstrably far from spherical If one assumes that it is a prolate ellipsoid the ratio of the long to the short axis as computed from Simha's equation (206) is 5 7 The shape of a protein molecule may also be expressed by the frictional constant, f/f_0, which can be computed by Perrin's equation (181) if one knows the ratio of the long to the short axis of a prolate ellipsoid of revolution Thus, f/f_0 for prolactin is calculated to be 1 29 (117)

The membrane diffusion method of Northrop and Anson (178) was employed to determine the diffusion coefficient of the hormone (108) After making the correction as suggested by Mehl (170), $D_{20} = 9.0 \times 10^{-7}$ cm ^{2}/sec From a combination of this constant with the frictional ratio, the molecular weight of prolactin may be estimated to be 22,000, which is in fair agreement with that obtained by osmotic pressure measurements

4 *Optical Rotation and Partial Specific Volume*

The optical rotation of prolactin solutions is found to be a linear function of the concentration (117) From the observed rotation of 1 0% solution at 25°C , the specific rotation of the lactogenic hormone is $-40.5°$ The partial specific volume of the hormone is calculated by

determining the density of the solution containing different weight fractions of the protein and found to be 0 721 This value is as expected for ordinary proteins

5 *Solubility*

Lactogenic hormone has some interesting solubility characteristics The pure hormone is soluble in absolute methyl or ethyl alcohol in the presence of a small amount of acid (71,121) It is extremely insoluble in water when no electrolytes are present The hormone isolated from ox glands has a solubility of 0 102 g /l at 7–8°C (133) In aqueous acid solution, prolactin is easily salted out in a low concentration of sodium chloride

TABLE V

PHYSICOCHEMICAL PROPERTIES OF LACTOGENIC HORMONE

Molecular Weight	
Osmotic pressure	26 500
Analytical data	25,000
Diffusion and Viscosity	22 000
Diffusion constant (D_{20})	$9\ 0 \times 10^{-7}$
Partial specific volume (V_1)	0 721
Viscosity coefficient	6 65
Dissymmetric constant (f/f_0)	1 29
Isoelectric point pH	5 73
Specific rotation	−40 5°
Solubility in water at 7 8°C , g /l	0 102

Table V summarizes the physicochemical properties of the lactogenic hormone

D DIFFERENCES IN OX AND SHEEP HORMONES

The hormone isolated from either ox or sheep pituitaries shows no difference in crop-sac stimulating potency Bischoff and Lyons (19) were unable to differentiate the ox and sheep hormone through the use of precipitin, anaphylaxis, or the Dale and Arthus reactions It was further found that the ox and sheep hormones can not be distinguished in electrophoretic experiments, they apparently migrate with the same mobility in buffers from pH 2 to 9 and have identical isoelectric points (129) The two hormones have the same stereochemical structure as judged by their optical rotation properties (117), there are also no differences in molecular weight and the content of tryptophan, arginine, cystine, and methionine (118 132) However, the hormone isolated from ox as contrasted with that isolated from sheep pituitary tissue can be differentiated by the following two methods

1 *Solubility Method*

The solubility method has been shown to be a sensitive test for distinguishing species specificity of proteins (112) Using this method, Li *et al* (134,135) found that ox and sheep lactogenic hormone are not identical proteins For instance, in 0 357 *M* sodium chloride solution at pH 2 25, the sheep hormone has a solubility of 0 506 g /l of the solvent at 25°C , whereas the solubility of the ox preparation is only 0 316 g In citrate buffer (1 *M*, pH 6 36), the ox hormone is more soluble than the sheep It is further shown that when sheep hormone is added to a saturated solution of the ox hormone, more protein is dissolved indicating that the two substances are not the same

The hormones isolated from these two species can also be demonstrated to be different entities by their behavior when salted out with sodium chloride Cohn (38) has shown that the solubility of a protein is defined by an equation of the form $\log S = \beta - K_s\mu$, where μ is the ionic strength/1000 g water S the solubility in g /l , and K_s and β constants It has been shown (133) that the hormone from ox or sheep glands has an almost identical K_s but different values for β are obtained when the salting out studies are made with sodium chloride in 0 01 *M* hydrochloric acid solution This means that in 0 01 *M* hydrochloric acid sheep hormone is more soluble than is the ox protein and that they are different proteins

In alcoholic solution, ox hormone is more soluble than the sheep (71)

2 *Tyrosine Method*

We have observed that the tyrosine content of ox lactogenic hormone is consistently higher than that of sheep, ox protein has 5 73% tyrosine, whereas sheep contains 4 53% (131,132) It is not likely that the difference in the solubility behavior of these two proteins can be completely explained by their tyrosine content and further determinations of other amino acids will be necessary to explain this phenomenon

E Analytical Data

1 *Elementary Composition*

The elementary analysis (121) of a pure lactogenic hormone preparation yields the following results C, 50 72%, H, 6 63%, N, 15 86%, S, 1 79%, P, nil Earlier investigators (51,160) had already found no phosphorus in purified lactogenic preparations The nitrogen content of the hormone was reported by White *et al* as 14 38%, 16 49%, and 16 84% (232,233)

2 *Distribution of Sulfur*

The hormone has no cysteine or sulfhydryl groups as shown by the nitroprusside, phosphotungstate, or iodine test (75,134), even when the protein is dissolved in denaturing agents, these tests are negative The presence of cystine in highly purified prolactin has been reported by Riddle and Bates (190) Fraenkel Conrat (75) employed both Sullivan (217) and phosphotungstate (72) methods for the quantitative determination of cystine and found that the pure hormone contains 3 0% cystine White, Bonsnes, and Long (232) reported a value of 3 36% as determined by the method of Sullivan and Hess (218) The cystine content is also shown to be 3 11% (118) in hydriodic acid hydrolyzates by the Baernstein procedure (6a) Since both the methods of preparing the hormone and the methods of analysis for cystine were not the same, the cystine values which have been reported should be regarded as in satisfactory agreement

Two methods have been used for the determination of methionine in lactogenic hormone one is a colorimetric (157) and the other a titration method (6a) The methods give results which are in agreement and the methionine content of the hormone may be assumed to be 4 31% (118)

If we take 4 31 and 3 11% as the methionine and cystine contents, respectively, the total sulfur in prolactin can be computed to be 1 76% It may be recalled that the sulfur content[8] was estimated as 1 79% when determined by the Carius method (132) Thus, the total sulfur in the lactogenic hormone is accounted for within the limits of error

3 *Tyrosine and Tryptophan Content*

As already mentioned, the lactogenic hormone isolated from ox glands has a higher tyrosine content (5 73%) than that from sheep glands (4 53%) The method used by Li *et al* (131) to obtain these values is the method of Lugg (150) White *et al* (232) reported a value of 5 51% for their ox preparation using the micro method of Folin and Marenzi (73) It may be mentioned that the presence of tyrosine in purified lactogenic preparations had been indicated by the experiments of earlier investigators (160 190)

The tryptophan content of lactogenic hormone seems to vary with the method of analysis used, the method of Lugg gives a value of 1 3% (133), on the other hand, a higher value (2 5%) is obtained by the glyoxylic acid procedure Recently we employed the *p*-dimethylaminobenzaldehyde reagent (219) and found that the tryptophan content of prolactin is 3 1% (121) By the method of Folin and Marenzi (73), White and

[8] A recent analysis of the sulfur content in lactogenic hormone was kindly performed by Dr T S Ma University of Chicago, and found again to be 1 79%

co-workers (232) reported 1 3% tryptophan in their prolactin preparation It is difficult to decide which of these values should be considered to represent the correct tryptophan content for lactogenic hormone We hope that future experiments with microbiological or isotope dilution methods may furnish the answer For the present, we may assume that prolactin contains 1 3% tryptophan

4 *Other Data*

The arginine and glutamic acid content of lactogenic hormone are shown to be 8 31% and 12 3%, respectively (116 132) The total basic and acid groups in the prolactin molecule have been determined by the dye technique and the hormone was found to contain 12 7 and 11 5 groups respectively, per 10,000 g protein (126) prolactin has 1 4% amid nitrogen (121) and 0 74% amino nitrogen (121)

A summary of the analytical data is presented in Table VI

TABLE VI

ANALYTICAL DATA* ON LACTOGENIC HORMONE

C	50 72	Cystine	3 11
H	6 63	Methionine	4 31
N	15 86	Tyrosine	
S	1 79	Ox	5 73
P	0 00	Sheep	4 53
Amino N	0 74	Tryptophan	1 30
Amide N	1 40	Arginine	8 31
Cysteine	0 00	Glutamic acid	12 30
		Groups/10 000 g protein	
		Acid	11 5
		Base	12 7

* Figures in per cent except where otherwise indicated

F REACTIONS WITH SPECIFIC REAGENTS

1 *Iodine*

In phosphate buffer of pH 7 0 iodine reacts only with the tyrosine groups in the prolactin molecule to form diiodotyrosine groups (134) This was established by the analysis of the iodine content of the iodinated hormone as compared with that calculated from the remaining free tyrosine When the hormone is completely saturated with iodine, the amount of iodine entering the protein is equal to that computed from the tyrosine content It is further shown that the isoelectric point of iodinated hormone has shifted from pH 5 73 to pH 4 7 This change corresponds to expectation, because Dalton *et al* (45) have shown that the pK

for phenolic groups in tyrosine is changed from 10.28 to 6.48 by iodination. In urea buffer solutions, the kinetic data suggest that there may be two types of tyrosine groups in the lactogenic hormone, one reacting with iodine at the same rate as pure tyrosine and the other more slowly.

When the iodinated hormone preparations are assayed in pigeons, a complete loss of crop sac stimulation is observed, indicating the essentiality of tyrosine groups for the hormonal action (135). In this respect, lactogenic hormone resembles pepsin and insulin; both of these substances (95,96) are inactivated by iodine and iodine reacts only with the tyrosine component of these two proteins.

2. *Reducing Agents*

The reactions of cysteine and thioglycolic acid with lactogenic hormone have been studied in some detail by Fraenkel-Conrat *et al.* (83). When the lactogenic hormone in solution is above 0.02%, treatment with a fortyfold amount of cysteine causes the formation of a precipitate and loss of biological activity. If the conditions of treatment are chosen so that they do not lead to precipitate formation, for example, the employment of a more dilute solution or a 6 to 8 *M* urea solution, no inactivation occurs. Even if the precipitate is formed, the lactogenic potency can be recovered by dissolving the precipitate under conditions which prevent autoxidation. When the amount of cysteine used is 200 times that of the hormone, a total inactivation is observed. Thioglycolic acid is more effective than cysteine in destroying lactogenic activity but the conditions necessary for the precipitation of the hormone are similar.

Fraenkel-Conrat investigated further the nature of the reducing groups in the hormone before and after treatment with thiol compounds (75). He stated that "treatment of lactogenic hormone with thiol compounds causes (a) the reduction of disulfide bonds to cysteine thiol groups, (b) the appearance of groups of unknown nature which reduce phosphotungstate and ferricyanide in neutral solution, and (c) the probable formation of stable addition compounds between the protein and the thiol compound." It is therefore difficult to explain the loss of lactogenic potency after treatment with cysteine or thioglycolic acid in terms of the interaction of specific groups in the protein with these reducing agents. Both the complexity of these reactions and the interesting behavior of the hormone with these particular reducing agents call for further investigations.

3. *Ketene*

Ketene has been known for some time as one of the most specific reagents for acetylation of the amino or phenolic groups in proteins.

It has become customary to assume that ketene reacts with the amino groups at a faster rate than with the phenolic hydroxyls Such an assumption has been applied in an earlier preliminary report on the ketenization of lactogenic hormone (136) and the authors concluded that the amino group is essential for crop-sac-stimulating activity We have recently reinvestigated the reactions of ketene with the lactogenic hormone (127) in more detail, with results which appear to be confirmatory of the previous conclusion

In phosphate buffer of pH 7 0, ketene reacts with the phenolic groups of the tyrosine residues in the hormone molecule more rapidly than with the amino groups If the reactions are carried out in acetate buffer of pH 4 0, the rates of acetylation of the amino and phenolic groups are identical When one compares assay results with the degree of acetylation of these two groups, it would appear that the decrease in crop-stimulating potency is due to coverage of the amino groups The authors have subjected an acetylated product to electrophoretic examination and found that it is quite homogeneous with respect to its electrochemical properties They further noted that the acetylated hormone migrates more slowly ($7\ 6 \times 10^{-5}$ cm ^{2}/v sec) than the untreated protein ($8\ 1 \times 10^{-5}$ cm ^{2}/v sec) in pH 2 2 buffer of 0 10 ionic strength

The employment of other reagents, such as phenyl isocyanate (23) and nitrous acid (128), has also demonstrated the essentiality of the amino group for the specific biological effects of the hormone

4 *Methyl Alcohol*

When lactogenic hormone is allowed to react with methyl alcohol in the presence of 0 025 to 0 1 *M* hydrochloric acid, the crop-sac stimulating action decreases progressively as the methoxyl content of the protein increases (126) It has recently been demonstrated (78) that groups in protein other than the carboxyl are not involved in the reaction with methyl alcohol and that the number of methoxyl groups that can be introduced into lactogenic hormone is slightly less than the number of carboxyl groups present Thus it would seem that the loss of lactogenic activity is due to the esterification of carboxyl radicals in the hormone molecule

The electrophoretic behavior of the esterified lactogenic hormone has also been investigated by Li and Fraenkel Conrat They found that the hormone derivative compares favorably in homogeneity with the untreated hormone and that the protein becomes more basic after esterification

There are thus far no indications of the existence of a prosthetic group or groups in the lactogenic hormone, the above discussions suggest

rather that the structural make up of the whole molecule is necessary for its physiological role At any rate modifications of the molecular structure hitherto studied tend to destroy the specific function of the hormone

G Effect of Various Agents

1 *Heat*

Lactogenic hormone solutions (1% at pH 7 6) are said to be capable of sterilization at 100°C for twenty minutes without loss of potency (154) In the absence of salt, the hormone shows little loss of potency after boiling for one hour at pH 8 0 or at 60°C for five hours, if salts are present, complete destruction may occur (190) At boiling water temperature, 0 04% hormone solutions of pH 1 to 9 are quite stable for 15 minutes treatment, at pH 11 and 13, a loss of lactogenic potency is observed When solutions of the same concentration at pH 1 to 13 are kept in a boiling water bath for thirty minutes, there is a great loss of biological activity (234) We have investigated the heat stability of the hormone further by maintaining 0 2% prolactin solution of pH 2 5 or pH 10 5 in 0 1% sodium chloride at 60°C for sixty minutes and found that no loss of activity is observed at the lower pH but a definite destruction occurs at pH 10 5 (121) From these results, it appears that the lactogenic hormone is more stable toward heat in acid than in alkaline solution

2 *Enzymes*

Lactogenic hormone activity is destroyed by pepsin and trypsin (11 160) White, Bonsnes and Long (232) confirmed these early results and found that "destruction of the biological activity of prolactin by pepsin takes place relatively early in the proteolytic process, in any case before decomposition into compounds that are not precipitated by the trichloroacetic acid"

3 *Denaturing Agents*

In the presence of 3 0 *M* urea, the relative viscosity of prolactin solution is greatly increased When the urea has been removed by dialysis, the original relative viscosity of the hormone returns (119) Thus, it is clear that a change of the hormone molecule occurs in urea solution and that this change is reversible as judged by viscosity measurements It may be recalled that the hormone activity is not reduced by urea if the denaturing agent is removed by dialysis before bioassays (132) It has furthermore been shown that the molecular weight of lactogenic hormone is not changed in urea solutions (132)

The effect of a detergent of the sodium alkyl aryl sulfonate type

(Nacconol) on the hormone has also been investigated (119) As is the case with urea, the relative viscosity of the hormone increases as the detergent concentration increases When these solutions were assayed in pigeons without the removal of the denaturing agent the lactogenic activity was diminished The loss of hormonal action may be due to the change in the molecular shape of the protein or to a hormone detergent complex formation A demonstration of the reversibility of the deter gent-protein combination and biological assay after removal of the detergent would be of great interest but this has not thus far been effected

III Thyrotrophic Hormone

Although the interrelationship between thyroid and pituitary in mammals had been known for many years it was due to the work of Smith that the existence of a thyrotrophic substance in the pituitary became evident By the technique of pituitary implants Smith (212, 214) restored the atrophic thyroid of hypophysectomized rats Similar results were obtained by Houssay *et al* (100) in the dog The first attempts to concentrate the thyrotrophic principle from pituitary extracts were carried out by Loeb and Bassett (145) and by Janssen and Loeser (102) The hormone has not yet been isolated in pure form, though highly purified preparations have been reported (36 84,231)

A Methods of Assay

The weight and histological development of the thyroid in guinea pigs, rats, and chicks have been employed as the index for the thyroid-stimulating potency of pituitary extracts

1 *Guinea Pigs*

The guinea pig was first introduced by Loeb and Bassett (145) and later widely employed by other investigators Junkmann and Schoeller (106) and others (5 98,109) utilized the histological changes in a young guinea pig's thyroid for the quantitative assay of the thyrotrophic hor mone Others (29,198) preferred the change in thyroid weight as an indication of the extent of thyrotrophic action It is generally agreed that histological signs of thyroid stimulation are more reliable than the changes in weight Furthermore, histological changes require smaller amounts of the hormone and occur more speedily Recently De Robertis and Del Conte (46) proposed a cytological technique which gives evidence of thyroid stimulation within thirty minutes after the administration of the hormone to young guinea pigs

2 *Chicks*

The test of thyrotrophic activity in chicks was first suggested by Smelser (210 211), it is based on the thyroid weight increase of one day old chicks The method has been generally accepted as more sensitive than that in guinea pigs (17,43 84,107) The strain of chicks is important Bates *et al* (13) observed that "chicks from the one source require 4 times as much thyrotropin to produce an equal amount of stimulation, as this is measured by an increase of thyroid weight, as do chicks from the other source' In Long s laboratory at Yale (36) similar difficulties were found in the use of the chick's thyroid weight as a measure of thyrotrophic potency However, Jorgensen and Wade (105) and Ciereszko (36) noted that histological changes in the chick's thyroid give more reliable and consistent results White Leghorn chicks three days old were injected subcutaneously daily for five days, on the sixth day the chicks were killed and autopsied The thyroids were fixed for histological examination (36)

3 *Rats*

The method suggested by Anderson and Collip (3) is not generally employed this depends upon the effect of the hormone on the meta bolic rate of hypophysectomized rats In this laboratory, we have employed the histological changes in the thyroid of hypophysectomized immature female rats as an indication of the degree of thyroid stimula tion The technique is highly sensitive and reliable in detecting minute amounts of thyrotrophic contamination in other pituitary hormone preparations The method was also employed by Chow, Greep, and van Dyke (34)

B Methods of Purification

The thyrotrophic potency of pituitaries of different species has been investigated by a number of workers McQueen Williams (158) found that the rat pituitary contains 7–9 times as much as that of the ox, while ox gland has greater thyrotrophic concentration than that of the guinea pig (50) Rowlands (196) showed that the content of the pituitary in this hormone decreases in the following order swine, dog, ox, horse and sheep The fact that ox pituitaries contain more thyrotrophic hormone than do sheep glands is confirmed by Jorgensen and Wade (105) It appears therefore that the best source of the hormone is constituted by swine and ox pituitaries

Both acid (84 106 111) and alkaline (3 70,146,198) extracts of pituitary tissue have been used for the preparation of thyrotrophic substance,

saline extraction (22,102) is also applicable The starting material may be either acetone desiccated or fresh pituitary glands To concentrate the thyrotrophic hormone from these extracts many fractionation techniques have been tried, such as alcohol or acetone (84,106), salt (70,84), or protein precipitants (17,36,105,111,146) It is of great interest to note that the thyrotrophic principle is soluble in the presence of some protein precipitating agents, namely, trichloroacetic acid, sulfosalicylic acid, lead acetate, etc This indicates that the hormone may possess a rather low molecular weight and would harmonize with the fact that ultracentrifugation does not concentrate the hormone (114,201)

Adsorption methods have also been employed in an effort to purify the thyrotrophic hormone Jorgensen and Wade (105) discovered that the hormone is selectively adsorbed by permutit at pH 4 5 and subsequently concentrated by precipitation with uranium acetate Repetition of these procedures does not appreciably further concentrate the hormone

In the following two methods one based on the differential precipitation by a protein precipitant and the other on salt fractionation are briefly described

1 *Yale Procedure (36)*

(*a*) Frozen whole ox pituitary glands were ground and extracted with 2% sodium chloride solution at pH 7 4 to 7 8. After centrifugation the supernatant was adjusted to pH 4 0 to 4 1 (*b*) The supernatant (pH 4 0 to 4 1) was mixed with an equal volume of acetone The precipitate formed was removed and more acetone was added up to 75% The 50–75% acetone-insoluble material was then dried by triturating with acetone (*c*) The acetone-dried precipitate was next extracted with distilled water The combined extracts were made to pH 9 0 and centrifuged The supernatant was adjusted to pH 7 0 and 5% lead acetate was added until precipitation was complete (*d*) 20% trichloroacetic acid was added to the lead acetate supernatant until the concentration became 8% (*e*) The trichloroacetic acid soluble fraction was dialyzed and concentrated by pervaporation After lyophilization the dried white solid is the purified thyrotrophic hormone From 1 kg of whole ox pituitaries about 400 to 500 mg of the preparation can be obtained

A total dose of 0 001 mg of the purified material causes histological changes in chick thyroids when injected over a period of five days The preparation is found free from growth, lactogenic, and gonadotrophic hormone Data on possible adrenocorticotrophic contamination have not been reported Ciereszko (36) stated that "preliminary electrophoretic examination of the product, as well as ultracentrifuge studies, suggests that it contains but one protein component" In an earlier report, White (231) showed that his preparation of thyrotrophic hormone (apparently the same one reported by Ciereszko) behaved like a single substance in one buffer solution It is, of course, desirable to study

electrochemical homogeneity in a protein in more than one buffer and at different pH values Neither White nor Ciereszko has as yet reported the solubility behavior of their thyrotrophic product

2 *California Procedure* (84)

(*a*) Acetone desiccated ox anterior pituitaries were extracted with 0 25% acetic acid–1% sodium chloride solution The supernatant was precipitated with an equal volume of acetone The precipitate formed was extracted with 1% sodium chloride solution (*b*) The saline extract was brought to 0 3 saturation with ammonium sulfate After removal of the precipitate the concentration of ammonium sulfate in the supernatant was increased to 0 6 saturation The 0 6 SAS precipitate was dissolved in water and again brought to 0 3 saturation with ammonium sulfate The supernatant was finally brought to 0 5 SAS (*c*) The 0 5 SAS precipitate was dissolved in water and dialyzed Acetone was next added to the dialyzed solution to 39% The precipitate formed was centrifuged off and the supernatant was poured into eight to ten times its volume of cold acetone The precipitate is the purified thyrotrophic hormone

The yield from 1 kg of acetone-desiccated ox pituitaries is about 2 6 g protein a total dose of 0 012 to 0 026 mg can cause a 33% increase in the thyroid weights of baby chicks over those of controls in six days The preparation has been carefully tested for contamination with other pituitary hormones it contains 0 025% lactogenic hormone, less than 3% adrenocorticotrophic hormone, about 1% growth hormone, 0 4% follicle stimulating hormone and about 10% interstitial cell stimulating hormone

It is difficult to compare the thyrotrophic potencies of the products purified by the two groups of investigators, for different methods of assay were employed If the thyroid weight and histology in the chick are of identical sensitivity as shown by Fraenkel Conrat *et al* (84), the Yale product would appear to be ten times as active as the California substance

C Physicochemical Properties

The nitrogen content in purified thyrotrophic preparations has been reported to be 12 37% (233), 12 6% (36), and 13 0% (84) It contains 3 5% hexose and 2 5% glucoseamine (84) White (233) showed that the purified hormone has 1 0% sulfur, while Ciereszko (36) gave a value of 1 2% No phosphorus was found in the preparations (36,231)

The hormone is highly soluble in water (36 84,231) It is not precipitated by sulfosalicylic acid (36,231) but is precipitated by phosphotungstic acid, picric acid uranium acetate, and mercuric chloride

White (231) reported the sedimentation constant of his preparation to be 1 0 Svedberg unit, the molecular weight was estimated to be approximately 10 000

Fraenkel Conrat *et al* (84) found that the thyrotrophic activity was destroyed by cysteine and ketene treatment

Using swine pituitary extract Chow *et al* (34) have studied the effects of various proteolytic enzymes on the thyrotrophic potency They found that the thyroid stimulating action is inactivated by crystalline chymotrypsin crystalline trypsin and crystalline pepsin Merck's trypsin may partially reduce the hormonal potency On the other hand papain does not destroy thyrotrophic activity

IV Adrenocorticotrophic Hormone (ACTH)

Since the first observation of Smith (214) it is well established that the size and function of the adrenal cortex is under the influence of the pituitary (101 220 222) In almost all species studied hypophysectomy causes adrenocortical atrophy and on the other hand hypophyseal implants or injections of pituitary extracts tend to enlarge the adrenal cortex and to induce hyperplasia of all three of its cell layers The substance in such extracts has been designated adrenotrophic or corticotrophic hormone Since the effect of hypophysectomy is confined to the cortex (220) it is proposed that the name adrenocorticotrophic hormone (ACTH) be employed

A Methods of Assay

The enlargement or histological change in the cortex of adrenals of normal and hypophysectomized animals has been taken as indicative of the adrenocorticotrophic activity of pituitary extracts Collip *et al* (41) suggested the removal of one adrenal from the hypophysectomized rat as the control the weight of which was compared with the weight of the remaining adrenal after the administration of hypophyseal extracts Reiss and co-workers (188) described a method based on the change in histological behavior of the adrenal of hypophysectomized rats Moon (171 172) employed 21-day or four-day old rats as test animals and increase in the adrenal weights was used as the index for adrenocorticotrophic potency The increment in adrenal weights in two-day old chicks has also been suggested (14) for assaying adrenocorticotrophic extracts Blumenthal (21) employed mitoses in the cells of the adrenal cortex of the guinea pig as a test for adrenocorticotrophic activity

Since it is known that adrenal hypertrophy in normal animals can be induced by many agents other than adrenocorticotrophic extracts (222) quantitative assays of ACTH must be carried out with animals after the removal of the hypophysis During the process of developing a procedure for the isolation of the adrenocorticotrophic hormone we have employed two assay methods for following the activity of adrenocorticotrophic frac

tions, these are based on the repair and maintenance of the adrenals of hypophysectomized rats (142,207) The methods have also been employed by Sayers *et al* (200) for the standardization of their ACTH preparations

1 *Repair Test*

The histological change in the adrenal cortex of rats after hypophysectomy is characterized primarily by the disappearance of lipides from most of the cortex Some lipides remain in the glomerulosa and scantily in the reticularis (171,188) The lipides also become large and more irregular in size in hypophysectomized animals The repair test (207) is based on the ability of adrenocorticotrophic hormone to amend these changes and cause a normal distribution of the lipides Females, 26–28 days of age are hypophysectomized and the adrenals hence allowed to regress for fourteen days, at which time injections are instituted and continued for four days, once daily intraperitoneally, followed by autopsy 96 hours after the first injection The adrenals are fixed in formol, cut as frozen sections, and stained with Sudan Orange The lowest effective amount of the hormone which gives recognizable beginnings in the redistribution of the cortical lipides in the adrenals of such animals is considered to be 1 unit of ACTH It may be mentioned that the adrenal weight does not significantly increase even after injection of 100 such units of the hormone The method is highly specific and sensitive It requires a very small amount of a pure preparation to obtain the beginning repair of the degenerated adrenals

2 *Maintenance Test*

This test is based on weight maintenance of the adrenals by injecting the hormone immediately after hypophysectomy Male rats (40 days old) are hypophysectomized and injected intraperitoneally (daily except Sunday) from the day of operation for fifteen days (thirteen injections) The adrenal weights of uninjected hypophysectomized animals regressed during this period from an average of 26 mg to a constant weight of approximately 12 mg The amount of ACTH which maintains the adrenals in such animals at 26 mg is 1 maintenance unit (207) We have found that the sensitivity of the maintenance test can be greatly increased if more than one injection daily is employed For instance, a 0 2-mg daily dose of the hormone will give adrenal weights of about 26 mg but the same daily dose when halved and injected twice daily will result in adrenal weights of 30 mg White (233) has shown that the sensitivity is also influenced by the strain of rat employed, he further showed that if adrenal weights are expressed per 100 g body weight, assays in different laboratories can be satisfactorily compared

B Methods of Isolation

Pituitary glands of sheep ox and swine have been used for the preparation of adrenocorticotrophic extracts Swine glands have been shown to have a higher concentration of ACTH than do sheep or ox glands (6) Besides pituitary tissues there are indications that the adrenocorticotrophic substance may be found in the serum of pregnant mares (87) and in female human urine (20 21)

In their method of preparing thyrotrophic extracts Collip *et al* (39 41) found that the adrenocorticotrophic activity is in the 75% acetone and 70%-ethanol soluble fractions Bates *et al* (14) reported that ACTH is soluble in 60% ethanol Therefore aqueous alcohol or acetone solutions have also been found useful in obtaining a potent adrenocorticotrophic extract (40 180 195)

It has been claimed by Anselmino *et al* (4) that the adrenocorticotrophic factor is diffusible through 8% acetic acid collodion membranes More recently Tyslowitz (224) has reported that dialyzates of a glacial acetic acid extract of acetone-dried swine pituitary tissue possessed a factor which caused an increase in the adrenal weights in hypophysectomized rats It would seem to be important to confirm these findings for they indicate that there may be another adrenocorticotrophic factor in swine pituitary—one which has a smaller molecular size

Perhaps the best starting material for the isolation of ACTH is the acid acetone extract of Lyons (153) It is particularly rich in two hormones only—the adrenocorticotrophic and lactogenic substances—but low in thyrotrophic gonadotrophic and growth activities Using this extract workers in two laboratories (124 142 199 200) have independently isolated adrenocorticotrophic hormone in pure form from sheep and swine glands by two different methods Their products are apparently identical in both biological and physicochemical properties In the field of pituitary hormone research it is almost proverbial that difficulties are encountered by any investigator who attempts to repeat the chemical procedure of another It is therefore particularly gratifying to note that the data obtained by these two laboratories are in complete agreement It may be added that Neufeld (175) has adopted a method similar to that described by Li *et al* (124 142) for the preparation of highly purified adrenocorticotrophic hormone

1 *Isolation of Swine ACTH*

The method of Sayers *et al* (199 200) employed whole swine pituitary glands as the starting material The procedure is based on the principle that both the hormone and its main contaminant (lactogenic hormone)

have low solubility at their isoelectric points It may be outlined in the following steps

(*a*) One kg of whole swine glands is extracted with acid acetone as described by Lyons (153) The extractable material is precipitated by increasing the acetone concentration to 92% The precipitate is extracted four times with 50 ml water The so-called crude prolactin" fraction is obtained by adding acetone to the combined aqueous extracts to 92% (*b*) The ' crude prolactin" is dissolved in water at pH 9 0 The precipitates formed by lowering the pH to 8 0, 6 6, and 5 4, successively, are removed by centrifugation The supernatant of pH 5 4 is brought to approximately 0 07 saturated ammonium sulfate After removal of the small amount of precipitate formed four volumes of acetone are added (*c*) The precipitate is dissolved in water and the solution is mixed with one half its volume of concentrated ammonium hydroxide After the ammoniacal solution is allowed to stand at room temperature for seven hours acetone is added to 90% (*d*) The precipitate is dissolved in water and dialyzed until salt free any precipitate formed during dialysis is discarded The solution is adjusted to pH 5 4 and centrifuged free of precipitate The pH is next lowered to 4 7, the precipitate is adrenocorticotrophic hormone

The product thus isolated has been subjected to electrophoretic experiments in buffers of four different pH values, indicating homogeneity of the preparation The sedimentation behavior of a 2% solution of the protein in a Beams analytical ultracentrifuge shows no evidence of the presence of a second component The purity of the hormone has not been examined from the standpoint of solubility studies When assayed in hypophysectomized male rats (total dose, 1 4 mg) and pigeon (total dose, 10 mg) the product has been shown to be free from growth, thyrotrophic gonadotrophic, and lactogenic activities It may be mentioned that the absence of alterations in the *weight* of the thyroid or testes were used by Sayers *et al* as reliable indications of the absence of thyrotrophic and gonadotrophic contaminants It is, however, well known that the weight changes in thyroid or testes of hypophysectomized animals after injecting thyrotrophic or interstitial-cell stimulating hormones are comparatively slight In our experience, histological studies should be used for indications of the absence or presence of these two contaminants in a purified preparation

2 *Isolation of Sheep ACTH*

Li, Evans, and Simpson (124,142) used fresh whole sheep glands as the starting material for obtaining the final pure protein—the adrenocorticotrophic hormone The procedure is as follows

(*a*) Sheep pituitaries are ground and extracted with acidified acetone The extract is precipitated in 90% acetone and dried (*b*) The precipitate is extracted with 0 10 *M* disodium phosphate the extract is next precipitated in 2 0 *M* ammonium sulfate The precipitate is dissolved in water and dialyzed until salt free (*c*) The dialyzed solution is brought to 0 54 *M* sodium chloride at pH 3 0, and centrifuged

The supernatant is again brought to 2.0 *M* ammonium sulfate (*d*) The precipitate is dissolved in water and to the solution is added half its volume of concentrated ammonium hydroxide The ammoniacal solution is allowed to stand at room temperature for four hours (*e*) The solution is then brought to 90% acetone The precipitate formed is dissolved in water and dialyzed against pH 7.5 phosphate buffer (*f*) Saturated ammonium sulfate is added to the dialyzed solution until a 1.65 *M* solution results The precipitate is dissolved in water and dialyzed again against pH 7.5 phosphate buffer (*g*) The dialyzed solution is put into a boiling water bath for two hours After heating ammonium sulfate fractionation is repeated (*h*) The final 1.65 *M* ammonium sulfate precipitate is dissolved in water and brought to 0.54 *M* sodium chloride and then to 1.36 *M* at pH 3.0 The precipitate coming down between 0.54 *M* and 1.35 *M* sodium chloride is the adrenocorticotrophic hormone

TABLE VII

YIELD IN EACH STEP IN ISOLATION OF ADRENOCORTICOTROPHIC HORMONE FROM ONE KILOGRAM FRESH WHOLE SHEEP GLANDS

Isolation step no (see text)	Fraction	Yield in N mg
b	Na_2HPO_4 extract	515
c	0.54 *M* NaCl supernatant	100
e	*Dialyzed soluble material of acetone* NH_4 ppt	65
g	Final 1.65 *M* $(NH_4)_2SO_4$ precipitate	37
h	Final NaCl ppt (ACTH)	11

The average yield in terms of nitrogen in each step is summarized in Table VII It may be noted that from 1 g of whole sheep glands approximately 70 mg of hormone could be isolated When the hormone is assayed in hypophysectomized male or female rats it is shown to contain less than 0.10% of follicle stimulating interstitial-cell stimulating, and thyrotrophic hormone as judged by histological changes It is also evident that the hormone is free from lactogenic and growth activities when assayed in pigeons and adrenalectomized rats By the method of Burn (27), the hormone contains less than 0.02% pressor substance Thus, it can be stated that the sheep ACTH isolated by the California method is completely free from other biologically active components except its own specific adrenocorticotrophic action The adrenocorticotrophic potency of the hormone as assayed by the maintenance test is summarized in Table VIII It will be seen that 0.2 mg of the pure substance satisfies the criterion for 1 Maintenance Unit of the hormone

The physicochemical purity of sheep ACTH has been examined by electrophoretic, ultracentrifugal, diffusion, and solubility studies When the hormone solution is electrolyzed in a Tiselius electrophoresis apparatus at seven different pH values it behaves as a single protein Solubility experiments in one solvent give a curve indicative of a single

component It must be admitted that solubility studies in other solvents should be carried out in order to fully sustain the belief that the product is a homogeneous substance

TABLE VIII

BIOASSAY OF SHEEP ADRENOCORTICOTROPHIC HORMONE IN FORTY DAY OLD HYPOPHYSECTOMIZED MALE RATS (MAINTENANCE TEST)

Daily dose, mg	No of rats	Body weight at autopsy, g	Adrenal weight, mg	Adrenal weight/100 g body wt , mg
0 0	19	127 5	12 0 ± 0 5*	9 4
0 02	14	107 8	15 6 ± 0 7	14 5
0 05	25	118 6	19 5 ± 1 0	16 4
0 10	15	117 9	22 3 ± 1 0	18 9
0 20	15	117 4	25 1 ± 0 9	21 4
1 00	10	107 8	40 1 ± 0 8	37 2

* Mean ± standard deviations

Sedimentation and diffusion studies of the hormone were made in the laboratory of J W Williams The protein is found to be homogeneous in ultracentrifugal experiments, but the results from one diffusion experiment carried out in a Lamm cell gave evidence of some inhomogeneity (28) It would be desirable to perform additional diffusion experiments with somewhat higher hormonal concentrations to establish homogeneity with respect to diffusibility

C PHYSICOCHEMICAL PROPERTIES

1 *Isoelectric Point*

The isoelectric point of adrenocorticotrophic hormone has been thought for some time to be at pH 6 0 to 6 5 (39,40,153) When the hormone was isolated in pure form, electrophoretic data revealed the fact that the isoelectric point of the sheep hormone is between pH 4 65 and 4 70 (124), while that of the swine protein lies between pH 4 7 and pH 4 8 (199) Although these two proteins have thus almost identical isoelectric points electrophoretic studies at other pH values may reveal differences with respect to electrochemical properties Unfortunately the data given by Sayers *et al* (199) do not specify the ionic strength of the buffers used, it is hence not possible to make satisfactory comparisons of the electrophoretic behavior of the two proteins

2 *Molecular Kinetic Data*

Burtner (28) obtained sedimentation and diffusion constants for the sheep adrenocorticotrophic hormone isolated by Li *et al* $S_{20} = 2\,08$ S,

$D_{20} = 10.4 \times 10^{-7}$ cm²/sec If the partial specific volume is assigned as 0.75, the molecular weight is computed to be 20,000, the frictional ratio, f/f_0 becomes 1.1, which gives as an approximate value 3/1 for the ratio of major to minor axis of the assumed unhydrated ellipsoidal molecule

Sayers *et al.* (199) gave the sedimentation constant of their swine hormone, $S_{20} = 2.04$ to 2.11 S. They reported no diffusion data but an estimated molecular weight of 20,000.

3. *Some Analytical Data*

The elementary analysis of a pure adrenocorticotrophic hormone isolated from sheep glands shows it to contain 46.3% C, 5.89% H, 15.65% N, and 2.30% S. There is no carbohydrate, phosphorus, or cysteine in the hormone. The hormone contains 1.93% methionine and 7.19% cystine (122), the content of these sulfur amino acids accounts for the amount of sulfur in the protein. Preliminary analysis (121) of tryptophan and tyrosine content by the method of Lugg (150) gives the following data: tyrosine 4.5%, tryptophan 1.0%.

4. *Solubility*

The adrenocorticotrophic hormone is very soluble in water and is only partly precipitated at its isoelectric point. In 2.5% trichloroacetic solution, it is almost completely precipitated (124). The hormone is readily precipitated from dilute solution by 20% sulfosalicylic acid and by 5% lead acetate solution (199). Bates *et al.* (14) reported that their adrenocorticotrophic principle had an appreciable solubility in 60 to 70% ethanol or acetone, even in the isoelectric region.

5. *Stability*

One of the remarkable properties of adrenocorticotrophic hormone is its resistance to heat treatment (124). When a hormone solution in a buffer of pH 7.5 is put in a boiling water bath for 120 minutes or longer, no loss of adrenocorticotrophic activity is observed. In 0.10 *M* hydrochloric acid, *0.2% hormone solution retains its biological potency when* it has been kept at 100°C. for sixty minutes, but when a hormone solution of the same concentration in 0.10 *M* sodium hydroxide is heated to 100°C. *for thirty minutes, the hormonal activity* is almost completely destroyed. In experiments conducted at 60°C. for sixty minutes, 0.2% adrenocorticotrophic hormone solution at pH 10.8 does not significantly lose its potency (121). The heat resistance of the adrenocorticotrophic activity of a purified preparation in acid solution has also been observed by Noble and Collip (177).

Table IX summarizes the physicochemical data for sheep and swine *adrenocorticotrophic hormone*

TABLE IX

PHYSICOCHEMICAL DATA[a] FOR SHEEP AND SWINE ADRENOCORTICOTROPHIC HORMONES[b]

Determinations	Sheep	Swine
C	46 35	50 64
H	5 89	6 23
S	2 30	2 33
N	15 65	15 47
P	0 00	
Carbohydrate	0 00	
Cystine	7 19	
Methionine	1 93	
Isoelectric point pH	4 65 to 4 70	4 70 to 4 80
Sedimentation constant, S_{20}	2 08	2 04 to 2 11
Diffusion constant $D_{20} \times 10^7$	10 40	
Molecular weight	20,000	20 000
Dissymmetric constant, f/f_0	1 1	

[a] See (28 122,124 199)

[b] Figures in per cent except where otherwise indicated

D EFFECT OF VARIOUS AGENTS

1 *Ketene*

When adrenocorticotrophic hormone solutions in pH 7 0 phosphate buffer were put in contact with a rapid flow of ketene for two, three, or five minutes, it was noted that one fifth of the phenolic groups in the hormone was acetylated while the loss of amino nitrogen was at most 50%, yet the adrenocorticotrophic activity continuously decreased (143) The authors conclude that the loss of hormonal action is at least partly due to the acetylation of the free amino groups

2 *Nitrous Acid*

If a 2 or 4% ACTH solution in pH 4 0 acetate buffer is allowed to react with 0 5 ml 4 *M* sodium nitrite solution for thirty minutes, the adrenocorticotrophic activity is completely abolished (143) The authors assume that if the behavior of adrenocorticotrophic hormone toward nitrous acid resembles that of pepsin (182), the free amino groups in the hormone are necessary for its biological activity

3 *Formaldehyde*

Further evidence that the primary amino groups of adrenocorticotrophic hormone are essential for its activity is obtained from a study of its behavior toward formaldehyde (143) When a mixture of 60 mg hormone and 1 ml 50% formaldehyde solution in 10 ml phosphate buffer (pH 7 0) was allowed to stand at room temperature for thirty minutes, a complete loss of hormonal activity resulted

4 *Iodine*

If a protein contains no cysteine, it takes up iodine through the tyrosine group (120) When adrenocorticotrophic hormone solutions were allowed to react with iodine in pH 7 0 buffer the iodine uptake corresponds approximately to the tyrosine content in the hormone and in the meantime, the biological potency is diminished (143) From these experiments, it was concluded that the tyrosine groups in adrenocorticotrophic hormone are also essential for its specific biological action

5 *Trypsin*

The effect of a commercial preparation of trypsin (Pfanstiehl) on adrenocorticotrophic hormone has been studied (124) It was noted that the adrenocorticotrophic activity was destroyed when 26% of the protein was digested by the enzyme However, no significant diminution of its activity was found when 18% of the hormone was hydrolyzed

E Hydrolysis with Pepsin

When adrenocorticotrophic hormone was digested with a commercial preparation of pepsin (Lilly) to an extent of 36–37% Li *et al* (124) found that the hormonal activity was unchanged as assayed by both the maintenance and repair tests Later experiments (121) confirm and extend these findings The new facts may be summarized as follows (*1*) The digestion with crude pepsin (Lilly) may be allowed to proceed until 50% of the protein hormone is hydrolyzed without loss of hormonal activity (*2*) If 60% of the hormone is digested with crystalline pepsin biological activity is not entirely destroyed (*3*) The trichloroacetic acid (5%) soluble fraction of the hormone after 50% hydrolysis with crude pepsin shows as much adrenocorticotrophic potency as the original material on the basis of total nitrogen content This indicates that the hydrolytic products possessing lower molecular weights—polypeptides—must possess the adrenal-stimulating activity (*4*) Analytical data suggest that these polypeptides may contain as few as eight amino acids

V. Growth Hormone

In 1921, Evans and Long (55) injected a simple saline extract of ox anterior pituitary substance into normal rats and found that the growth of these animals was accelerated and the final body weight heavier than in litter mate controls Later experiments of Smith (213) with hypophysectomized rats showed that the growth of these animals was resumed on administration of the same anterior pituitary extracts Thus, the existence of a growth hormone in these extracts was indicated The isolation of the hormone in pure form has only recently been achieved (123,125)

A Methods of Assay

Three methods for the standardization of growth hormone potency are currently employed Two of them are based on the body growth of normal or hypophysectomized female rats and have been widely used in growth hormone research The third method has been adequately explored only recently and appears to be specific, it depends on the rapid increase in width of the uncalcified cartilage of the epiphyseal disc of a long bone of a young hypophysectomized animal

1 *Body Growth of Normal Female Rats*

Though use of normal "plateaued" female rats, five to six months of age and weighing from 220 to 280 g for the assay of growth hormone fractions was first suggested by Evans and Simpson (58), such rats, although they have reached growth stasis, can be induced to grow readily by the administration of the growth hormone In actual practice, a hormonal preparation to be assayed was injected intraperitoneally or subcutaneously into groups each of which consisted of at least ten normal plateaued female rats for twenty days (seventeen injections) A dose level that causes an increase in body weight of from 40 to 60 g in this period is most reliably chosen for such an assay, a unit is defined as the daily dose producing a total weight increase of 40 g in twenty days (62) It was found that a straight line relationship exists between the logarithm of the dose level and the response in body growth (24,77) and that the slope of this line is essentially the same for both cruder and more purified preparations (166) However, the slope of the line increases with increasing periods of injection, up to fifteen days (166) For this reason, a shorter period of injection than fifteen days is not advisable

Although normal "plateaued" female rats possess the advantages of being more resistant to the toxic effects of crude preparations and in being more easily available, their notably lower sensitivity to the hormone and the longer period of injections required have limited their use

2 *Body Growth of Hypophysectomized Female Rats*

When hypophysectomized rats are employed for growth tests, it is essential that they should be rigorously standardized as to strain, sex, age, weight, physiological condition, and postoperative period if reliable results are to be obtained The animals are not used for assay unless evidence for complete hypophysectomy has been obtained The following criteria are generally used to ascertain the completeness of the operation limitation of body weight gain to 7 g in the first eight days after hypophysectomy, impairment of body tonus, maintenance of infantile hair, and the final exploration of the sella at autopsy

For routine assay, a group of ten hypophysectomized female rats, 28–30 days of age at operation and ten–fourteen days postoperative, receive nine intraperitoneal injections in ten days A unit is defined as the daily dose which causes an average weight gain of 10 g in this period (62) For the highest degree of accuracy it may be necessary to use a fifteen-day injection period (166) When the weight gain is plotted against the logarithm of the dose, a straight line is also obtained (144,166) Very high levels of dosage are beyond the dose range which gives the straight line relationship For this reason only that part of the curve or line should be employed in an assay

It is well recognized that hypophysectomized rats are more sensitive in their response to growth hormone than are normal "plateaued" female rats Marx *et al* have shown that "the hypophysectomized rats showed almost a double relative response with only about $\frac{1}{3}$ of the relative dose as was given to the normal rats" (166) Chou *et al* (31) noted that hypophysectomized male and female rats are equally responsive to injections of growth promoting extracts It is not advisable to use test animals more than once to obtain results of the highest accuracy The animals which have been employed for a previous assay are neither as uniform nor as sensitive in their growth response (24,42,166)

3 *Tibia of Hypophysectomized Rats*

The influence of the hypophysis on skeletal growth has been known for some time (48,94,205), but the specific action of growth hormone on the epiphyseal cartilages of the long bones of hypophysectomized animals has been described only recently (85,184) Kibrick *et al* (108) concluded that these effects were as follows "*hypophysectomy rapidly initiates* a loss in the dimensions of the epiphyseal plate, despite the fact that growth of cartilage and bone may continue for a short time in the young animal *after the removal of the pituitary* This loss in thickness reflects the initial disturbance of the equilibrium that normally exists between

chondrogenesis and oesteogenesis Administration of growth hormone rapidly restores the dimensions of the cartilage plate by stimulating first chondrogenesis and then oesteogenesis until an equilibrium is re-established " Based on these premises, Evans *et al* (60) proposed a new method for bioassay of the pituitary growth hormone using the width of the proximal epiphyseal cartilage of the tibia in hypophysectomized rats as the index of hormonal stimulation This method was found to be approximately three times as sensitive as that dependent upon body growth, when compared with other pituitary hormonal preparations it was found highly specific (168) for the growth hormone

The procedure requires the use of hypophysectomized female rats, 26 to 28 days of age at operation, twelve to thirteen days postoperative The hormone tested was administered intraperitoneally once daily for four days The autopsy was made 24 hours after the last injection, the right tibia of each animal was dissected, split with a safety razor blade and fixed in neutral formol Routine staining with silver nitrate and sodium thiosulfate was used The uncalcified portion of the epiphysis was then measured under the microscope with a calibrated eye piece micrometer As was found in the body growth of hypophysectomized or normal rats in their response to growth hormone, here also a plot of the width of the uncalcified cartilage against the logarithm of the hormone dose gives a straight-line relationship (60)

B Method of Isolation

Growth promoting extracts of pituitary glands may be obtained either with saline (22,55) or alkaline solutions Such alkaline solutions are made from NaOH (57), $Ba(OH)_2$, (52), $Ca(OH)_2$ (169) or NH_4OH (70) From these extracts, Teel (221), van Dyke and Wallen-Lawrence (226) were early able to show that the growth hormone activity may be precipitated out as a globulin in the presence of sodium sulfate This fact led Evans *et al* (62) to use ammonium sulfate as a precipitating agent for the hormone They found that growth promoting activity is in the precipitate which occurs between 0 2 and 0 5 SAS, but the precipitate, though containing the growth hormone, was not freed from other active components In 1940, Fraenkel-Conrat *et al* (77) employed cysteine to inactivate these contaminants and some further increase in growth potency was achieved with ammonium sulfate fractionation or isoelectric precipitation (167)

Adsorption methods have also been employed for the purification of growth hormone Collip *et al* (42) used calcium sulfate to adsorb the hormone, whereas Dingemanse and Freud (47) employed activated carbon (Norit) as the adsorbent and phenol as the solvent of the hormone

It is not established that these methods selectively separated the growth hormone from all its contaminants

Success in the isolation of the growth hormone in pure form has only recently been attained after long-continued effort in this laboratory (123,125) The isolation method may be briefly summarized in the following steps

(*1*) The dissected anterior lobes of ox pituitaries are ground and dried with chilled acetone (−10°C) The acetone-dried powder is extracted with calcium hydroxide solution at pH 11 5 for 24 hours, the pH is then lowered to 8 7 by adding carbon dioxide gas (*2*) The supernatant is brought to 2 0 *M* ammonium sulfate The precipitate formed is dissolved in water and the ammonium sulfate precipitation repeated The final precipitate is dialyzed until free of salt (*3*) The insoluble material formed during dialysis is next dissolved in water at pH 4 0 The solution is brought to 0 10 *M* sodium chloride The resulting precipitate is removed and the supernatant is brought to 5 0 *M* sodium chloride The sodium chloride fractionation is repeated twice (*4*) The final 5 0-*M* sodium chloride precipitate is dissolved in water and dialyzed until salt free The thoroughly dialyzed solution is adjusted to pH 5 7 to 5 8, the precipitate is centrifuged off and the supernatant made alkaline and adjusted to pH 8 7 to 8 8 The clear pH 8 8 fluid is then brought to 1 65 *M* ammonium sulfate at pH 7 0 The pH and ammonium sulfate fractionation is repeated twice (*5*) The dialyzed solution of the final 1 65 *M* ammonium sulfate precipitate is first adjusted to pH 5 7 to 5 8 and then pH 8 7 to 8 8 as above finally the solution is precipitated at pH 6 8 to 6 9 in the absence of ammonium sulfate This isoelectric precipitation is repeated twice The final pH 6 8 precipitate is the growth hormone

A nearly crystalline preparation of growth hormone could easily be obtained by cooling its warm supersaturated solution after the removal of some precipitates at pH 7 0 Fig 1 gives a photomicrograph of such preparations

C Criteria of Purity

1 *Biological Test*

The hormone isolated by the procedure just described contains no, or extremely low amounts of, biologically active contaminants When a total dose of 10 mg is injected subcutaneously into month-old squabs (*four days*), *there appears no lactogenic potency* A *total dose of 5 mg* in hypophysectomized female rats in four days gives no histological evidence of the presence of adrenocorticotrophic, thyrotrophic, or gonadotrophic hormones [9]

[9] However, when the preparation is injected in old hypophysectomized rats (over one year postoperative) for 39 days with a total dose of 5 8 mg , results (15) indicated that it may contain a minute quantity of thyrotrophic substance It may be agreed that the most sensitive tests for the purity of a biologically active protein are biological assay methods, if the contaminating substances are physiologically active.

2 *Diffusion*

The homogeneity of the hormone with respect to molecular size has been examined by diffusion experiments using the sintered glass disc technique of Northrop and Anson (178) It was found that the amount of diffused nitrogen/unit time was practically constant and that there was no difference in growth potency of the protein solution after or before the diffusion process

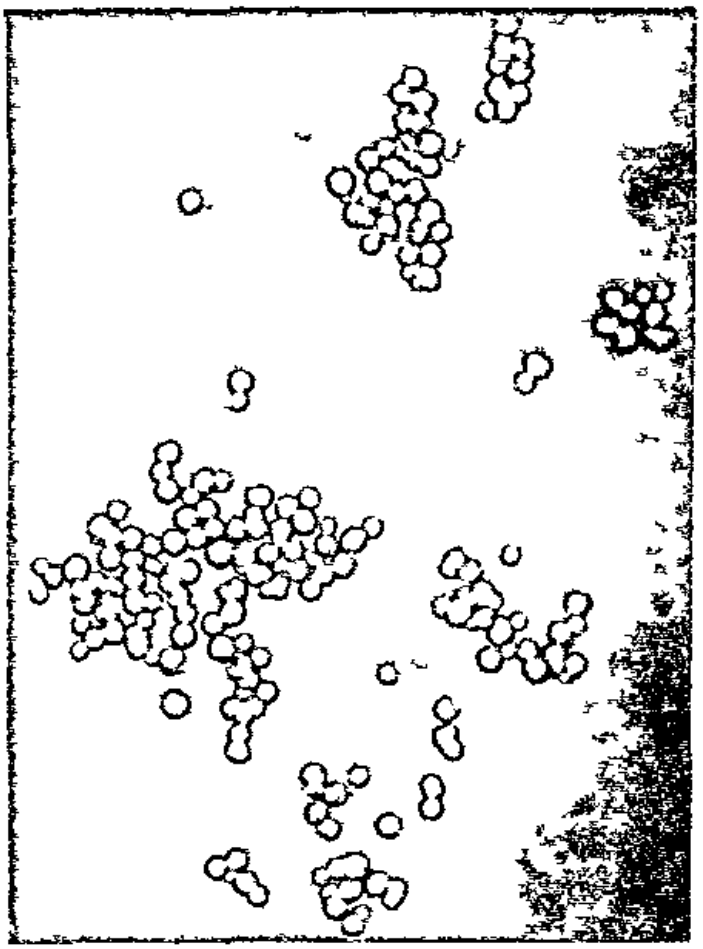


FIG 1 —The growth hormone (×250)

3 *Electrophoresis*

When a 1% growth hormone solution is subjected to electrophoretic studies in a Tiselius apparatus (223) with the scanning method of Longsworth (148), the hormone migrates as a single component in three buffers of pH 4 0 4 95 and 9 60 Fig 2 presents a few electrophoresis patterns of such experiments

In one experiment, the protein which appeared in three different sections of the electrophoresis cell (the parent solution was submitted

for 120 minutes at pH 4 0 to a potential gradient of about 6 v /cm) was recovered separately and no significant difference in growth potency of the three fractions could be detected The fact that no electrophoretic separation of the hormone could be achieved indicates that the preparation is homogeneous as to its electrochemical properties

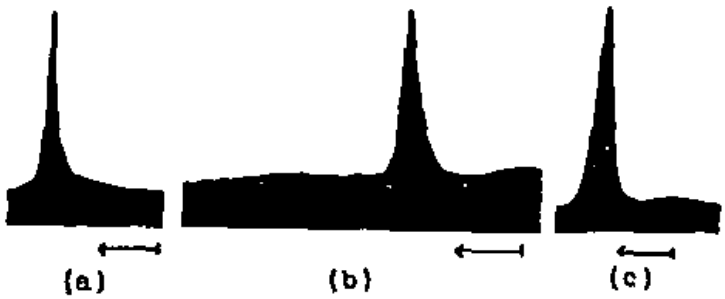


FIG 2 —Electrophoretic patterns of the ascending boundary of growth hormone preparations (a) acetate buffer of pH 4 0, 120 minutes electrolysis (b) acetate buffer of pH 4 95, 540 minutes electrolysis (c) barbiturate buffer of pH 9 60 All buffers of 0 1 ionic strength, 1 5°C

4 *Solubility*

Since melting points of proteins can not be determined without decomposition, the ordinary methods for determining purity in organic compounds are not suitable for substances of protein nature Northrop and colleagues have used extensively the solubility technique (97) to detect impurities in their crystalline enzyme preparations It is now generally agreed that the solubility test is the best physical method to establish the purity of protein preparations

Three solvents were used to study the solubility behavior of the growth hormone In each instance, the solubility of the hormone was constant after the appearance of the solid phase indicating the singularity of the preparation The solubility curves are given in Fig 3

Ultracentrifugal studies of the hormone have not been made, in view of the uniformity of the diffusion rate, the hormone is most probably homogeneous with respect to molecularity Furthermore, no instance is known in which a protein behaves like a pure substance in solubility experiments but shows heterogeneity in ultracentrifugal tests

D Biological Potency

When the growth hormone preparations were assayed in hypophysectomized female rats for growth-promoting activity, it was noted that 0 01 mg daily for ten days (nine injections) caused an average of 10-g increase in body weight Table X summarizes the assay results with different dose levels of growth hormone The biological potency of the

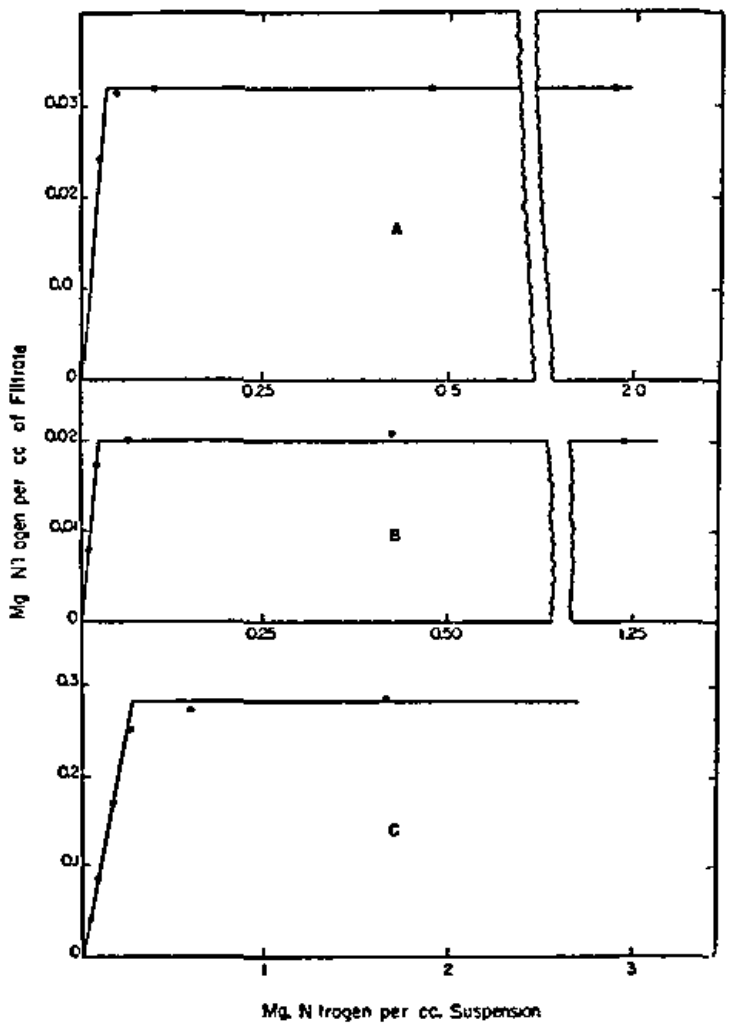


FIG 3.—Solubility curves of the growth hormone in various solvents at 5°. (A) solvent, distilled water, pH 7.1, (B) solvent, 4.8 *M* NaCl in 0.07 *M* phosphate buffer, pH 5.7, (C) solvent, 3.8 *M* NaCl in 0.07 *M* phosphate buffer, pH 6.4

TABLE X

ASSAY OF GROWTH HORMONE IN HYPOPHYSECTOMIZED FEMALE RATS

Daily dose, mg	No. of rats	Av. growth in 10 days, g	Daily dose, mg	No. of rats	Width of uncalcified cartilage	
					Mean, μ	Difference from control, μ
0.10	36	19.2	0.05	6	344	189
0.05	21	18.3	0.03	8	296	141
0.02	11	11.0	0.01	8	220	65
0.01	18	10.0	0.00	7	155	0

hormone is further standardized by its action on the epiphyseal cartilage of hypophysectomized animals The results are also included in Table X, it is evident that a 0 010-mg daily dose for four days is needed to cause an increase of 50% in the width of the uncalcified portion of the proximal epiphyseal cartilage over that of the control

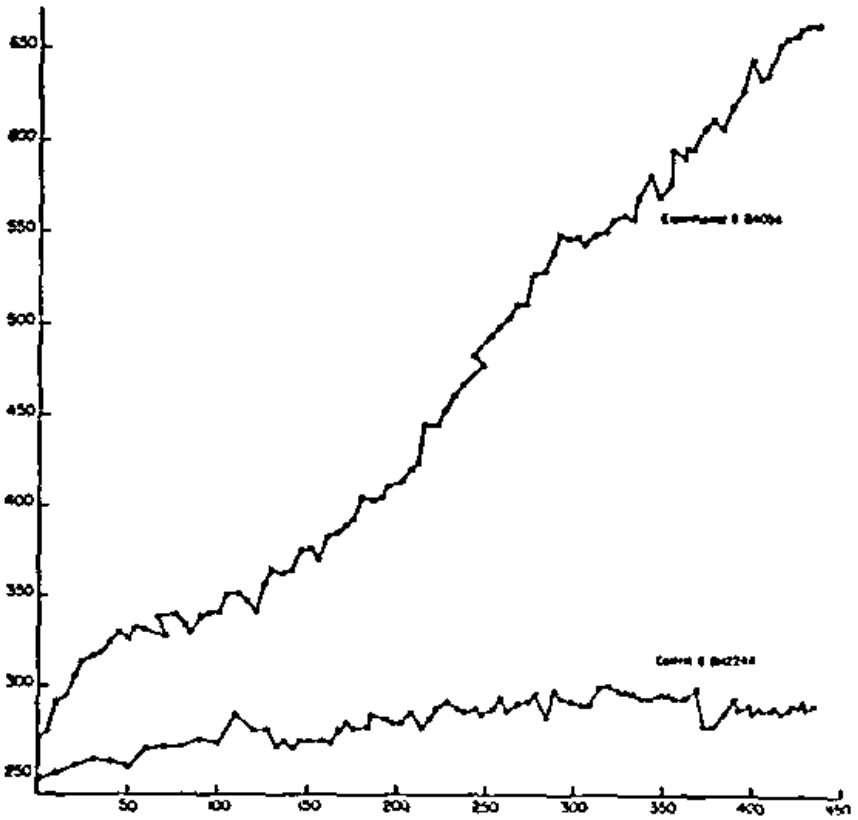


FIG 4 —Growth curves of normal plateau female rats The experimental animal received daily 0 40 mg growth hormone the first 23 days, 0 60 mg daily the next 68 days, 1 0 mg daily the next 33 days, 1 5 mg daily the next 115 days, 2 0 mg daily the last 193 days no injections on Sundays The control rat received no injections

There has been some discussion as to the ability of growth hormone to cause *continuous* growth in normal "plateaued" or in hypophysectomized rats To sum up the situation Long (147) has said "In the first experiments reported by Evans and Long the rats were injected daily for as long as 8 to 13 months with a crude alkaline extract and although growth was not as rapid in the late period as at first, nevertheless it was continuous throughout the period of injections Later attempts to repeat this experiment, even in the same laboratory, showed that, after an initial period of brisk growth, the animals became refractory to the extract and even lost some of the weight they had gained The same result was also obtained in hypophysectomized animals but even more

discouraging was the fact that partial purification of the extract did not correct this decreased responsiveness "

We have recently injected the growth hormone into normal "plateaued" female rats for 435 days, the daily dose increased gradually from 0 4 mg to 2 0 mg It was found that the animals gained weight continuously although the growth rate became somewhat slower in the later period of the injections Fig 4 presents the growth curves of a

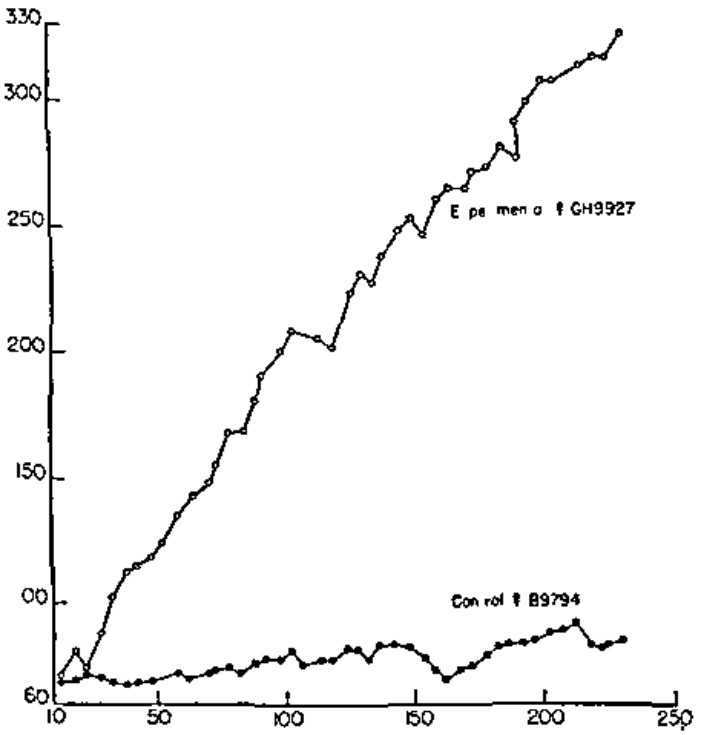


FIG 5 —Growth curves of hypophysectomized female rats The experimental animal received 0 10 mg daily growth hormone the first 140 days and then increased the daily dose to 0 20 mg no injections on Sundays The control received no injections

typical injected rat and a control It can be seen that the body weight of the rat increased from 270 g to 664 g , whereas the control gained only 42 g

A similar experiment with hypophysectomized female rats, 26–28 days of age and twelve to fourteen days postoperative, also indicates that the pure growth hormone induces continuous growth in such animals and there is no sign of refractoriness with over 200 days of injections The daily dose required in this experiment is much less than that for normal rats, it changes from an initial dose of 0 10 mg to 0 20 mg in the later period of the experiment Though the experiments with hypophysectomized animals are still in progress, we wish to present at this time the growth rate of an injected rat and its control in Fig 5 It is

apparent that the pure growth hormone can cause continuous body growth in both hypophysectomized and normal rats if the dose of the hormone is increased proportionately with the size of the animal

E Physicochemical Properties

1 *Isoelectric Point and Molecular Weight*

From electrophoretic mobility data, the isoelectric point of growth hormone is estimated to be pH 6 85 This value is considerably higher than that found for lactogenic, adrenocorticotrophic, and sheep interstitial-cell-stimulating hormones

The osmotic pressure measurement was employed for the determination of the molecular weight When the protein is dissolved in 0 40 *M* or 0 80 *M* ammonium sulfate solution at pH 6 64 to 7 00, the calculated molecular weight from the observed osmotic pressures is found to be 44,250 This value can also be derived from analytic, viscosity, and diffusion data However, the molecular weight as determined by ultracentrifugal studies has yet to be obtained

2 *Analytical Data*

Elementary analysis of pure growth hormone preparations gives the following results C, 46 35%, H, 7 07%, S, 1 30%, N, 15 50% (Kjeldahl), 15 65% (Dumas) There is no phosphorus or carbohydrate The amino nitrogen as determined by the Van Slyke manometric technique is 0 76%, the amide nitrogen 1 20%

The tyrosine and tryptophan content of the hormone are 4 30 and 0 92%, respectively as estimated by the method of Lugg (150) The glutamic acid was determined by Lewis and Olcott (116) using a microbiological method and amounts to 13 40% The hormone contains no cysteine The cystine and methionine content of the hormone are shown to be 2 25 and 3 06%, respectively (122) The sulfur in the protein is completely accounted for by these amounts of cystine and methionine If one assumes that the hormone contains two molecules of tryptophan, four molecules of cystine, nine molecules of methionine, ten molecules of tyrosine, forty molecules of glutamic acid, and eighteen atoms of sulfur, an average computed molecular weight for growth hormone is found to be 43,575 This value is in good agreement with that obtained by the osmotic pressure method

3 *Diffusion and Viscosity (121)*

The diffusion constant of the hormone was determined in an electrophoresis cell as suggested by Longsworth (149) When the corrections

for the solvent and temperature were made, the diffusion constant D_{20} (water) became 7.15×10^{-7} cm^2/sec

The viscosity of the hormone solutions was measured in a capillary viscometer as described by Neurath *et al* (176) A plot of the protein concentration against the observed relative viscosity gives the constant for the specific viscosity, $(\eta/\eta_0 - 1)1000/cV_1 = 7.64$ From this constant the ratio of major to minor axis of the hormone protein is shown to be 6/1 when Simha's equation (206) is used This indicates that the growth hormone molecule is more elongated than the adrenocorticotrophic and lactogenic hormones

We have computed the molecular weight from these data and a value of 39 300 is obtained It is apparent that this value is in fair agreement with that estimated from osmotic pressure and analytic data

Table XI summarizes the known physicochemical data of anterior hypophyseal growth hormone

TABLE XI

PHYSICOCHEMICAL DATA OF GROWTH HORMONE

C %	46.30	Cysteine %	0.00
H %	7.07	Cystine %	2.25
S %	1.30	Methionine %	3.06
N (Dumas) %	15.65	Glutamic acid %	13.40
N (Kjeldahl) %	15.50	Molecular weight	
Amino N %	0.76	Osmotic pressure	44 250
Amide N %	1.20	Analytic data	43 575
No of acid groups/10^4 g protein	9.80	Diffusion viscosity	39 300
No of base groups/10^4 g protein	13.40	Isoelectric point pH	6.85
Tyrosine %	4.30	Diffusion constant $D_{20} \times 10$	7.15
Tryptophan %	0.92	Partial specific volume V_1	0.76
		Viscosity coefficient	7.64
		Dissymmetric constant f/f_0	1.31

F EFFECT OF VARIOUS AGENTS

1 *Effect of Proteolytic Enzymes*

When dilute solutions of growth hormone were incubated at 37°C with trypsin or pepsin the growth potency was greatly reduced Such results are to be expected if the protein is the hormone

2 *Effect of Heat*

It has been generally believed that the growth hormone is a thermolabile substance (56 204) Using the pure hormone it was found that the hormone activity was completely lost at pH 4.0 7.5, and 8.9 in boiling water temperature for ten minutes When an intermediate temperature

was applied for one hour, it was observed that the hormonal solution (0.02% at pH 7.0) remained perfectly clear up to 60°, turbidity began at 70° and flocculent precipitation occurred at 80°. On the other hand, the growth potency was not significantly reduced at 60°C but it was destroyed at 70° and 80° when the solutions were cooled and assayed in hypophysectomized rats. This indicates that the hormonal activity cannot be separated from the protein and that the protein isolated is the hormone. It was further found that the growth potency was destroyed at pH 3.1 when the protein solution was kept at 60°C for one hour. Under similar conditions the hormone remains unchanged at pH 10.5. This substantiates the belief that the growth hormone is more stable in alkali than in acid solutions.

3. *Effect of Urea*

When 1% growth hormone solution in pH 7.0 6.66 *M* urea stood for 24 hours at room temperature, it was shown that the biological potency did not change.

4. *Effect of Nitrous Acid and Ketene (121)*

Growth hormone (8 mg.) was dissolved in 2 ml. pH 4.0 acetate buffer and 0.25 ml. 4 *M* sodium nitrite was added. The solution was allowed to stand at 22°C., after thirty minutes, it was neutralized and assayed by the tibia test. It was found that the hormonal activity was completely destroyed. Since nitrous acid probably only reacts with the free amino groups in the protein under these conditions, it may be concluded that primary amino groups are essential for the growth potency.

We have also employed ketene to react with growth hormone. A 1.2% growth hormone solution in pH 4.0 acetate buffer was treated with a rapid flow of ketene at 0°C. for thirty minutes. Afterward the acetylated hormone was recovered by dialysis and lyophilized. Analytical results indicated that the amino groups were covered to the extent of 75%, while 35% of the phenolic groups were acetylated. Both tibia and body growth tests in hypophysectomized female rats showed that the product contained no growth promoting activity. Since both amino and phenolic groups react with ketene, it is difficult to say which of these groups is responsible for the loss of the biological action. However, when the acetylated hormone in pH 11.0 solution was allowed to stand at 22°C. for sixty minutes, the Folin phenol color was completely recovered indicating that the phenolic groups became free. When the acetylated product was assayed after such treatment no indications of the recovery of hormone activity were secured. Thus, it is certain that the loss of growth activity was at least partly due to acetylation of the amino

groups in the hormone It may therefore be concluded that the free amino groups are essential for the growth-promoting action

VI. Summary

We are certain at the present time that there are at least six hormones in extracts of the anterior lobe of the pituitary Four of them have been isolated in pure form, *viz*, the lactogenic, interstitial-cell stimulating, adrenocorticotrophic, and growth hormones The physicochemical properties of these hormones are distinctly different among themselves Tables XII and XIII summarize the known physicochemical characteristics of these proteins

TABLE XII

PHYSICOCHEMICAL PROPERTIES OF ICSH, ACTH, LACTOGENIC AND GROWTH HORMONES

Determination	ICSH		ACTH		Lactogenic hormone		Growth hormone (ox)
	Sheep	Swine	Sheep	Swine	Sheep	Ox	
Molecular Weight Osmotic pressure	40 000				26,500	26,500	44,250
Sedimentation		100,000	20 000	20 000		32,000	
Diffusion constant, $D_{20} \times 10^7$			10 4		9 0	7 5	6 6
Sedimentation constant S	3 6	5 4	2 8	2 04 to 2 11		2 65	
Isoelectric Point pH	4 6	7 45	4 65 to 4 70	4 70 to 4 80	5 73	5 73	6 85
Partial specific volume V_1					0 721		0 76
Viscosity coefficient					6 65		7 64
Dissymetric constant f/f_0			1 1		1 29		1 31

Biologically the six known hormones can be classified into two groups the first group may be called the gonadotrophic hormones, which includes follicle-stimulating, interstitial cell-stimulating, and lactogenic hormones, while the growth, adrenocorticotrophic, and thyrotrophic principles belong to the so-called metabolic hormones Chemically, these hormones may be divided into two types of proteins glycoproteins (thyrotrophic, follicle-stimulating and interstitial-cell-stimulating hormones) and simple proteins (growth, adrenocorticotrophic, and lactogenic

hormones) From their solubility characteristics, the growth and lactogenic hormone may be said to be water-insoluble proteins whereas the follicle-stimulating, interstitial-cell-stimulating, and thyrotrophic hormones are highly soluble in water

TABLE XIII

SOME ANALYTICAL DATA* OF ICSH, ACTH, LACTOGENIC, AND GROWTH HORMONES

Determination	ICSH		ACTH		Lactogenic hormone		Growth hormone (Ox)
	Sheep	Swine	Sheep	Swine	Sheep	Ox	
C		49 37	46 35	50 64	50 72	51 50	46 35
H		6 83	5 89	6 23	6 63	6 92	7 07
N	14 20	14 93	15 65	15 47	15 86	16 50	15 65
S			2 30	2 33	1 79	2 00	1 30
Amino N					0 74		0 76
Amide N					1 40		1 20
Cysteine	0 0		0 0	0 0	0 0	0 0	0 0
Cystine			7 19		3 11	3 4	2 25
Methionin			1 93		4 31		3 06
Tyrosine	4 5				4 53	5 7	4 30
Tryptophan	1 0	3 8			1 30	1 3	0 92
Glutamic acid					12 30		13 40
Arginine					8 31		
Groups/10,000 g							
Acid					11 5		9 8
Base					12 7		13 4
Mannose	4 5	2 8	0 0		0 0		0 0
Hexoseamine	5 8	2 2	0 0		0 0		0 0

* Figures in per cent except where otherwise indicated

Though these hormones can easily be separated from the extracts of anterior pituitary tissue, it is far from certain that identical substances are actually secreted into the blood stream by the hypophysis Research on hormones isolated from the blood stream itself must furnish light on such problems

Finally, it cannot be overlooked that other hormones beside the six just mentioned may be present in and secreted by the pituitary gland The discovery of new physiologically active substances will utilize a thorough knowledge of the biological reactions of the individual *pure* hormones and their combinations as compared with implants or injections of crude extracts of *whole anterior pituitary substance* Such biological responses, like those at present known, will have to be studied as to their effects, for example, upon enzyme systems Hardly a beginning in such inquiries has as yet been made

REFERENCES

1 Abramowitz, A A , and Hisaw, F L *Endocrinology* **25**, 633 (1939)
2 Abramson, H A Electrokinetic Phenomena in Biology and Medicine Chemical Catalog Co , New York, 1934
3 Anderson, E M , and Collip, J B *J Physiol* **82**, 11 (1934)
4 Anselmino, K J , Harold, L , and Hoffman, F *Klin Wochschr* **12**, *1436 (1933)*
5 Aron, M *Compt rend soc biol* **123**, 250 (1936)
6 Astwood, E B , and Tyslowitz, R *Federation Proc* **1**, 24 (1942)
6a Baernstein, H D *J Biol Chem* **106**, 451 (1934), **115**, 24, 33 (1936).
7 Bates, R W , and Riddle, O *J Pharmacol* **55**, 365 (1935)
8 Bates, R W , and Riddle, O *Proc Soc Exptl Biol Med* **34**, 847 (1936)
9 Bates, R W , and Riddle, O *ibid* **44**, 505 (1940)
10 Bates, R W , and Riddle, O *Endocrinology* **29**, 702 (1941)
11 Bates, R W , Riddle, O , and Lahr, E L *Proc Soc Exptl Biol Med* **31**, 1223 (1934)
12 Bates, R W , Riddle, O , and Lahr, E L *Am J Physiol* **125**, 722 (1939)
13 Bates, R W , Riddle, O , and Lahr, E L *Endocrinology* **29**, 492 (1941)
14 Bates, R W , Riddle, O , and Miller, R A *ibid* **27**, 781 (1940)
15 Becks, H , Simpson, M E , Evans, H M , Li, C H , and Asling, C W *Anat Record* (in press)
16 Bergman, A J , and Turner, C W *J Biol Chem* **118**, 247 (1937)
17 Bergman A J , and Turner, C W *Endocrinology* **24**, 656 (1939)
18 Bischoff, F *J Biol Chem* **134**, 641 (1940)
19 Bischoff, H W , and Lyons, W R *Endocrinology* **25**, 17 (1939)
20 Blumenthal, H T *ibid* **27**, 477 (1940)
21 Blumenthal, H T *J Lab Clin Med* **30**, 428 (1945)
22 Bonsnes, R W , and White, A *Endocrinology* **26**, 990 (1940)
23 Bottomley, A C , and Folley, S J *Nature* **145**, 304 (1940)
24 Bulbring, E *Quart J Pharm Pharmacol* **11**, 26 (1938)
25 *Bull Health Org* **7**, 887 (1938)
26 *ibid* **8**, 909 (1939)
27 Burn, J H *Quart J Pharm Pharmacol* **4**, 57 (1931)
28 Burtner, E *J Am Chem Soc* **65**, 1238 (1943)
29 Chance, M R A , Rowlands, I W , and Young, F G *J Endocrinology* **1**, 239 (1939)
30 Chen, G , and van Dyke, H B *Proc Soc Exptl Biol Med* **40**, 172 (1939)
31 Chou, C , Chang, C , Chen, G , and van Dyke, H B *Endocrinology* **22**, 322 (1938)
32 Chow, B F *ibid* **30**, 657 (1942)
33 Chow, B F *Ann N Y Acad Sci* **43**, 309 (1943)
34 Chow, B F , Greep, R O , and van Dyke, H B *J Endocrinol* **1**, 440 (1939)
35 Chow, B F , van Dyke, H B , Greep, R O , Rothen, A , and Shedlovsky, T *Endocrinology* **30**, 650 (1942)
36 Ciereszko, L S *J Biol Chem* **160**, 585 (1945)
37 Coffin, H C , and van Dyke, H B *Science* **93**, 61 (1941)
38 Cohn, E J *Ann Rev Biochem* **4**, 136 (1935)
39 Collip, J B *J Mt Sinai Hosp* **1**, 28 (1934)
40 Collip, J B *J Am Med Assoc* **115**, 2073 (1940)
41 Collip, J B , Anderson, E M , and Thomson, D L *Lancet* **2**, 347 (1933)

42 Collip, J B, Selye, H, and Thomson, D L *Proc Soc Exptl Biol Med* **30**, 544 (1933)
43 Cope, C L *J Physiol* **94**, 358 (1938)
44 Corner, G W *Am J Physiol* **95**, 43 (1930)
45 Dalton, J B, Kirk, P L, and Schmidt, C L A *J Biol Chem* **88**, 589 (1930)
46 De Robertis, E, and Del Conte, E *Rev soc argentina biol* **20**, 88 (1944)
47 Dingemanse, E, and Freud, J *Acta Brevia Néerland Physiol Pharmacol Microbiol* **5**, 109 (1935)
48 Dott, N M, and Fraser, J *Quart J Exptl Physiol (Suppl)* **13**, 107 (1923)
49 Ehrhardt, K, and Voller, H F *Endokrinologie* **22**, 19 (1939)
50 Emerson, K, Jr *Johns Hopkins Hosp Bull* **60**, 358 (1937)
51 Evans, E I *Am J Physiol* **119**, 303 (1937)
52 Evans, H M, Cornish, R E, and Simpson, M E *Proc Soc Exptl Biol Med* **27**, 101 (1929)
53 Evans, H M, Fraenkel Conrat, H L, Simpson, M E and Li C H *Science* **89**, 249 (1939)
54 Evans, H M, Korpi, K, Simpson, M E, Pencharz, R I, and Wonder D H *Univ Calif Pub Anat* **1**, 255 (1936)
55 Evans, H M, and Long, J A *Anat Record* **21**, 62 (1921)
56 Evans, H M, Meyer, K, and Simpson, M E *Mem Univ Calif* **11**, 59 (1933)
57 Evans, H M, and Simpson, M E *Anat Record* **29**, 356 (1925)
58 Evans, H M, and Simpson, M E *Am J Physiol* **98**, 511 (1931)
59 Evans, H M, Simpson, M E, Lyons, W R, and Turpeinen, K *Endocrinology* **28**, 933 (1941)
60 Evans, H M, Simpson, M E, Marx, W, and Kibrick, E A *ibid* **32**, 13 (1943)
61 Evans, H M, Simpson, M E, Tolksdorf, S, and Jensen, H *ibid* **25**, 529 (1939)
62 Evans, H M, Uyei, N, Bartz, Q R, and Simpson, M E *ibid* **22**, 483 (1938)
63 Fevold, H L *Cold Spring Harbor Symposia Quant Biol* **5**, 93 (1937)
64 Fevold, H L *J Biol Chem* **128**, 83 (1939)
65 Fevold, H L *Endocrinology* **24**, 435 (1939)
66 Fevold, H L *ibid* **28**, 33 (1941)
67 Fevold, H L *Ann N Y Acad Sci* **43**, 321 (1943)
68 Fevold, H L, Hisaw, F L, Hellbaum, A, and Hertz, R *Am J Physiol* **104**, 710 (1933)
69 Fevold, H L, Hisaw, F L, and Leonard, S L *Am J Physiol* **97**, 291 (1931)
70 Fevold, H L, Lee, M, Hisaw, F L, and Cohn, E J *Endocrinology* **26**, 999 (1940)
71 Fleischer, J A *J Biol Chem* **147**, 525 (1943)
72 Folin, O, and Marenzi, A D *ibid* **83**, 103 (1929)
73 Folin, O, and Marenzi, A D *ibid* **83**, 89 (1929)
74 Folley, S J, Dyer, F J, and Coward, K H *J Endocrinology* **2**, 179 (1940)
75 Fraenkel Conrat, H L *J Biol Chem* **142**, 119 (1942)
76 Fraenkel Conrat, H L, Li, C H, Simpson, M E, and Evans, H M *Endocrinology* **27**, 793 (1940)
77 Fraenkel-Conrat, H L, Meamber, D L, Simpson, M E, and Evans, H M *ibid* **27**, 605 (1940)
78 Fraenkel-Conrat, H L, and Olcott, H S *J Biol Chem* **161**, 259 (1945)
79 Fraenkel Conrat, H L, Simpson, M E, and Evans, H M *ibid* **130**, 246 (1939)

80 Fraenkel Conrat, H L, Simpson, M E, and Evans, H M *Ann Facultad Medicina, Montevideo*, XXV (1940)
81 Fraenkel Conrat, H L, Simpson, M E, and Evans, H M *Science* **91**, 363 (1940)
82 Fraenkel Conrat, H L, Simpson, M E, and Evans, H M *Proc Soc Exptl Biol Med* **45**, 627 (1940)
83 Fraenkel Conrat, H L, Simpson, M E, and Evans, H M *J Biol Chem* **142**, 107 (1942)
84 Fraenkel Conrat, J, Fraenkel Conrat, H L, Simpson, M E, and Evans, H M *ibid* **135**, 199 (1940)
85 Freud, J, Levie, L H, and Kroom, D B *J Endocrinology* **1**, 56 (1939)
86 Gardner, W U, and Turner, C W *Agr Exptl Sta Missouri Research Bull* **196** (1933)
87 Golla, Y M L, and Reiss, M *J Endocrinology* **3**, 5 (1942)
88 Greep, R O *Anat Record (Suppl)* **73**, 2, 23 (1939)
89 Greep R O, van Dyke, H B, and Chow, B F *J Biol Chem* **133**, 289 (1940)
90 Greep R O, van Dyke H B, and Chow, B F *Anat Record* **78**, 88 (1940)
91 Greep R O van Dyke, H B, and Chow, B F *Proc Soc Exptl Biol Med* **46**, 644 (1941)
92 Greep R O, van Dyke, H B, and Chow, B F *Endocrinology* **30**, 635 (1942)
93 Gurin, S *Proc Soc Exptl Biol Med* **49**, 48 (1942)
94 Handelman, M B, and Gordon, E F *J Pharmacol* **38**, 349 (1930)
95 Harington, C R, and Neuberger, A *Biochem J* **30**, 810 (1936)
96 Heriot, R M *J Gen Physiol* **20**, 335 (1937)
97 Herriot, R M *Chem Revs* **30**, 413 (1942)
98 Heyl, J G, and Laqueur, E *Arch intern Pharmacodynamie* **49**, 338 (1935)
99 Hoffman, F *Z Gynak* **61**, 35 (1937)
100 Houssay, B A, Biasotti, A, and Magdalena, A *Compt rend soc biol* **110**, 142 (1932)
101 Ingle D J *Endocrinology* **31**, 419 (1942)
102 Janssen S and Loeser, A *Arch exptl Path Pharmakol* **163**, 517, 1931
103 Jensen H Simpson, M E, Tolksdorf, S, and Evans, H M *Endocrinology* **25**, 67 (1939)
104 Jensen H Tolksdorf, S, and Bamman, F *J Biol Chem* **135**, 791 (1940)
105 Jorgensen M N and Wade, N J *Endocrinology* **28**, 406 (1941)
106 Junkmann K and Schoeller, N *Klin Wochschr* **11**, 1176 (1932)
107 Kabak J M and Liapin, C W *Bull Biol Med Exper* **5**, 334 (1938)
108 Kibrick E A, Becks, H, Marx, W, and Evans H M *Growth* **4**, 347 (1941)
109 Kippen A A and Loeb L *J Pharmacol* **54**, 246 (1935)
110 Kupperman H S, Elder, W H, and Meyer, R *Endocrinology* **29**, 23 (1941)
111 Lambie C G and Trikojus, V M *Biochem J* **31**, 843 (1937)
112 Landsteiner K and Heidelberger, M *J Gen Physiol* **6**, 131 (1923)
113 Langecker H and Schenk, F *Med Klin* **32**, 1104 (1936)
114 Levin L Smelser, G K, and Moore, D H *Endocrinology* **30**, 171 (1942)
115 Levin, L and Tyndale, H H *ibid* **21**, 619 (1937)
116 Lewis J C and Olcott, H S *J Biol Chem* **157**, 265 (1945)
117 Li, C H *ibid* **146**, 633 (1942)
118 Li C H *ibid* **148**, 289 (1943)
119 Li C H *ibid* **155**, 45 (1944)
120 Li, C H *J Am Chem Soc* **67**, 1065 (1945)
121 Li C H Unpublished data

122 Li, C H *Federation Proc* **5**, Pt 2, 144 (1946)
123 Li, C H, and Evans, H M *Science* **99**, 183 (1944)
124 Li, C H, Evans, H M, and Simpson, M E *J Biol Chem* **149**, 413 (1943)
125 Li, C H, Evans, H M, and Simpson, M E *ibid* **159**, 353 (1945)
126 Li, C H, and Fraenkel Conrat, H L Unpublished data
127 Li, C H, and Kalman, A. *J Am Chem Soc* **68**, 285 (1946)
128 Li, C H, Lyons, W R, Simpson, M E, and Evans, H M *Science* **90**, 376 (1939)
129 Li, C H, Lyons, W R, and Evans, H M *J Am Chem Soc* **62**, 2925 (1940)
130 Li, C H, Lyons, W R, and Evans, H M *J Gen Physiol* **23**, 433 (1940)
131 Li, C H, Lyons W R, and Evans H M *J Biol Chem* **136**, 709 (1940)
132 Li, C H, Lyons, W R, and Evans, H M *ibid* **140**, 43 (1941)
133 Li, C H, Lyons, W R, and Evans, H M *J Gen Physiol* **24**, 303 (1941)
134 Li, C H, Lyons, W R, and Evans, H M *J Biol Chem* **139**, 43 (1941)
135 Li, C H, Lyons, W R, Simpson, M E, and Evans, H M *Science* **91**, 530 (1940)
136 Li, C H, Simpson, M E, and Evans, H M *ibid* **90**, 140 (1939)
137 Li, C H, Simpson, M E, and Evans, H M *J Biol Chem* **131**, 259 (1939)
138 Li, C H, Simpson, M E, and Evans H M *Endocrinology* **27**, 803 (1940)
139 Li, C H, Simpson, M E, and Evans, H M *Science* **92**, 355 (1940)
140 Li, C H, Simpson, M E, and Evans H M *J Am Chem Soc* **64**, 367 (1942)
141 Li, C H, Simpson, M E, and Evans, H M *J Biol Chem* **146**, 627 (1942)
142 Li, C H, Simpson, M E and Evans, H M *Science* **96**, 450 (1942)
143 Li, C H, Simpson M E, and Evans, H M *Arch Biochem* **9**, 259 (1946)
144 Light, A E, De Beer, E J, and Cook, C A *Proc Soc Exptl Biol Med* **44**, 192 (1946)
145 Loeb, L, and Bassett, R B *ibid* **27**, 490 (1930)
146 Loeser, A *Arch exptl Path Pharmakol* **166**, 693 (1932)
147 Long, C N H *Ann N Y Acad Sci* **43**, 383 (1943)
148 Longsworth, L G *J Am Chem Soc* **61**, 529 (1939)
149 Longsworth, L G *Ann Acad N Y Sci* **41**, 267 (1941)
150 Lugg, J W H *Biochem J* **32**, 775 (1938)
151 Lyons, W R. *Anat. Record (Suppl.)* **64**, 31 (1935)
152 Lyons, W R. *Cold Spring Harbor Symposia Quant Biol* **5**, 198 (1937)
153 Lyons, W R *Proc Soc Exptl Biol Med* **35**, 645 (1937)
154 Lyons, W R *Endocrinology* **28**, 161 (1941)
155 Lyons, W R., and Catchpole, H R. *Proc Soc Exptl Biol Med* **31**, 299 (1933)
156 Lyons, W R, and Page, E *ibid* **32**, 1049 (1935)
157 McCarthy, T E, and Sullivan, M X *J Biol Chem* **141**, 871 (1941)
158. McQueen-Williams, M *Proc Soc Exptl Biol Med* **32**, 1050 (1935)
159 McQueen-Williams, M *ibid* **33**, 406 (1935)
160 McShan, W H, and French, H E *J Biol Chem* **117**, 111 (1937)
161 McShan, W H, and Meyer, R K *ibid* **126**, 361 (1938)
162 McShan, W H, and Meyer, R K *Proc Soc Exptl Biol Med* **40**, 701 (1939)
163 McShan, W H, and Meyer, R K *J Biol Chem* **135**, 473 (1940)
164 McShan, W H, and Meyer, R K *Proc Soc Exptl Biol Med* **61**, 57 (1946)
165 McShan, W H, and Turner, C W *ibid* **34**, 50 (1936)
166 Marx, W, Simpson, M E, and Evans, H M *Endocrinology* **30**, 1 (1942)
167 Marx, W, Simpson, M E., and Evans, H M *J Biol Chem* **147**, 77 (1943)
168. Marx, W, Simpson, M E, and Evans, H M *Proc Soc Exptl Biol Med* **55**, 250 (1944)

169 Meamber, D L, Fraenkel Conrat, H L, Simpson, M E, and Evans, H M *Science* **90**, 19 (1939)
170 Mehl, J W Cited by Oncley (179)
171 Moon, H D *Proc Soc Exptl Biol Med* **35**, 649 (1937)
172 Moon, H D *ibid* **43**, 42 (1940)
173 Nelson, W O *Anat Record* **60**, 69 (1934)
174 Nelson, W O, and Pfiffner, J J *Proc Soc Exptl Biol Med* **25**, 1 (1930)
175 Neufeld, A H *ibid* **54**, 90 (1943)
176 Neurath, H, Cooper, G R, and Erickson, J O *J Biol Chem* **138**, 411 (1941)
177 Noble, R L, and Collip, J B *Endocrinology* **29**, 934 (1941)
178 Northrop J H, and Anson, M L *J Gen Physiol* **12**, 543 (1928–29)
179 Oncley, J L *Ann N Y Acad Sci* **41**, 121 (1941)
180 Perla, D *Proc Soc Exptl Biol Med* **34**, 751 (1936)
181 Perrin, F *J Phys radium* **7**, 1 (1936)
182 Philpot, J St L, and Small, P A *Biochem J* **32**, 542 (1938)
183 Pirie, N W *Biol Rev Cambridge Phil Soc* **15**, 377 (1940)
184 Ray, R D, Evans, H M, and Becks, H *Am J Path* **17**, 509 (1941)
185 Reece, R P, and Turner, C W *Proc Soc Exptl Biol Med* **34**, 402 (1936)
186 Reece, R P, and Turner, C W *ibid* **35**, 60 (1936)
187 Reece R P, and Turner, C W *Univ Missouri Research Bull* **266**, 1 (1937)
188 Reiss, M, Balint, J, Oestreicher, F, and Aronson, V *Endokrinologie* **18**, 1 (1936)
189 Reiss, M, and Haurowitz, F *Z ges exptl Med* **68**, 371 (1929)
190 Riddle, O, and Bates, R W In E Allen, Sex and Internal Secretions 2nd ed Williams & Wilkins, Baltimore, 1939, p 1088
191 Riddle O, Bates, R W, and Dykshorn, S W *Proc Soc Exptl Biol Med* **29**, 1211 (1932)
192 Riddle, O, Bates, R W, and Dykshorn, S W *Anat Record (Suppl)* **54**, 25 (1932)
193 Riddle, O, Bates, R W, and Dykshorn, S W *Am J Physiol* **105**, 191 (1933)
194 Riddle, O, Bates, R W, Lahr, E L, and Moran, C S *ibid* **116**, 128 (1936)
195 Rosen S H and Marine, D *Proc Soc Exptl Biol Med* **41**, 647 (1939)
196 Rowlands I W *J Physiol* **88**, 298 (1936)
197 Rowlands, I W. *Quart J Pharm Pharmacol* **10**, 216 (1937)
198 Rowlands, I W, and Parkes, A S *Biochem J* **28**, 1829 (1934).
199 Sayers G, White, A, and Long, C N H *J Biol Chem* **149**, 425 (1943)
200 Sayers, G, White, A, and Long C N H *Proc Soc Exptl Biol Med* **52**, 199 (1943)
201 Severinghaus A E, Levin, L, and Chiles, J A *Endocrinology* **23**, 285 (1938)
202 Shedlovsky, T *Ann N Y Acad Sci* **43**, 253 (1943)
203 Shedlovsky, T, Rothen, A, Greep, R O, van Dyke, H B, and Chow, B F *Science* **92**, 178 (1940)
204 Shipley, R A *Endocrinology* **31**, 629 (1942)
205 Sieberberg, M *Proc Soc Exptl Biol Med* **32**, 1423 (1935)
206 Simha, R *J Physiol Chem* **44**, 25 (1940)
207 Simpson, M E, Evans, H M, and Li, C H *ibid* **33**, 261 (1943)
208 Simpson, M E, Li, C H, and Evans, H M *Endocrinology* **30**, 977 (1942)
209 Simpson, M E, Li, C H, and Evans, H M *ib d* **35**, 96 (1944)
210 Smelser, G K *Proc Soc Exptl Biol Med* **37**, 388 (1937)
211 Smelser G K *Endocrinology* **23**, 429 (1938)

212 Smith, P E *Anat Record* **32**, 221 (1926)
213 Smith, P E *J Am Med Assoc* **88**, 158 (1927)
214 Smith, P E *Am J Anat* **45**, 205 (1930)
215 Smith, P F *Univ Pennsylvania Bicentennial Conference* **19**, 1941
216 Stricker, P and Grueter F *Compt rend soc biol* **99**, 1978 (1928)
217 Sullivan, M X *U S Pub Health Service Pub Health Repts* **44** 1600(1929)
218 Sullivan, M X and Hess W C *U.S Pub Health Service Pub Health Repts* (Suppl) 86 (1930)
219 Sullivan, M X, Milone H S, and Everitt E L *J Biol Chem* **125**, 471 (1938)
220 Swann, H G *Phys Rev* **20**, 493 (1940)
221 Teel, H M *Science* **69**, 403 (1929)
222 Tepperman, J, Engel F L and Long C N H *Endocrinology* **32**, 373 (1943)
223 Tiselius, A *Trans Faraday Soc* **33**, 542 (1937)
224 Tyslowitz, R. *Science* **98**, 225 (1943)
225 van Dyke, H B The Physiology and Pharmacology of the Pituitary Body Chicago I, 1936 II, 1939 Also P E Smith in E Allen Sex and Internal Secretions Williams & Wilkins Baltimore 1939, Chapter XVI
226 van Dyke H B, and Wallen Lawrence Z *J Pharmacol* **40**, 412 (1930)
227 van Dyke, H B, and Wallen Lawrence Z *ibid* **47**, 163 (1933)
228 Wallen Lawrence, Z *ibid* **51**, 263 (1934)
229 West, E, and Fevold, H L *Proc Soc Exptl Biol Med* **44**, 446 (1940)
230 White, A *Ann N Y Acad Sci* **43**, 341 (1943)
231 White, A In The Chemistry and Physiology of Hormones Science Press, Lancaster, Pa, 1945, p 1
232 White, A., Bonsnes R W, and Long, C N H *J Biol Chem* **143**, 447 (1942)
233 White, A, Catchpole, H R., and Long C N H *Science* **86**, 82 (1937)
234 Witschi, E *Endocrinology* **27**, 437 (1940)
235 Young, F G *J Endocrinol* **1**, 339 (1939)
236 Zondek, B, and Aschheim S *Klin Wochschr* **6**, 248 (1927)

Chapter XV

Hormonal Control of Mammary Growth

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For any exposition of the part played by hormones in the development and functioning of the normal mammary gland we find ourselves presented with an array of established or partly attested scientific facts, collected mostly during the last two decades and providing a body of evidence already sufficiently unequivocal to be of some value to the clinician and to warrant optimism regarding their future application to the practical problems of dairy husbandry. On the other hand there are still issues of fundamental importance which are held in doubt, or around which rival theories spread an enlivening controversy. Broadly, two main aims may be attributed to research in lactational physiology: to find the hormonal mechanisms governing the development of the mammary gland itself, and to determine the influences controlling the secretion and ejection of milk from glands so formed. A useful and unartificial classification of our present knowledge may well be based on a separate consideration of these complementary paths of research, and the present chapter has been written with this plan in mind.

I. Morphology of the Mammary Gland

It is essential for a proper evaluation of studies on the growth and differentiation of mammary tissue to consider first the typical morphological structures of which the glandular parenchyma is composed and to relate certain well-defined stages in the development of these

structures to corresponding phases in the reproductive life of the animal The literature dealing with the microscopic anatomy of the gland has been ably summarized by Turner (155), whose publication, although dealing primarily with development in the bovine, ranges extensively to provide a most valuable contribution to comparative anatomy and incidentally deserves to be far more widely known

This author, while stressing the variations to be found in the development and gross anatomy of the mammae of different species, testifies to the uniformity of the histological structure of the parenchyma Whether one considers species as distinct as man and the marsupial *Dasyurus viverrinus* or the cow and the rabbit, the same basic type of tissue structure is to be observed though in the monotremes or egg-laying mammals—we may instance the duck-billed platypus—the apparatus has unusual characteristics and offers in many respects a more primitive version of the general form There are two structures of major importance the alveoli (acini), which are the secretory organs, and the ducts which act as channels through which the secretion passes to the external orifice of the gland Questions of the endocrine influence on mammary growth largely resolve themselves into observations on the proliferation or regression of these structures in response to different stimuli

The duct system may be compared with a complicated arborescence, at the ends of whose smallest members, by analogy the twigs, are to be found the bulbous alveoli Secretion from the alveoli flows from smaller to larger ducts, which in high-yielding species such as the cow derive their names—intralobular, interlobular, intralobar, and interlobar—from their internal or interconnective relationships with morphologically recognizable aggregations of alveoli known as lobules and lobes The latter units are given their individual character by the surrounding connective tissue, thinner in the case of the lobules, which are composed of alveoli, than in the lobes, which are composed of lobules In smaller laboratory animals such as the rat, a more usual duct nomenclature is that distinguishing the major primary duct and secondary, tertiary, quaternary, and terminal ducts arising from its subsequent, and usually dichotomous branchings The terminal and lateral buds on the smaller ducts of the undeveloped gland represent the anlagen of the future lobules and each secondary duct with its bud like growths constitutes a potential lobe Histologically the alveoli and all but the finest ducts may be distinguished from one another by their single cell and double-cell epithelia, while the ducts, again excluding the finest, have an outer layer of connective tissue of which there is no counterpart of comparable thickness enveloping the alveoli It is contended that some secretory activity is a function of those cells of the fine terminal ducts which lie

closest to the alveoli and a transition to a single cell epithelium has been demonstrated in these areas as has also the presence of a relatively simple Golgi apparatus (168) These facts suggest secretory powers shared only in negligible degree if at all by the double cell epithelia lining the larger ducts (134) while the report of pronounced vacuolization of the cytoplasm of duct cells during late pregnancy in rats (135) also lends support to the theory of terminal duct secretion Jeffers (60) has furthermore commented upon certain parallelisms to be noted in the degeneration of alveolar and some duct epithelial cells in the lactating rat This question is more fully discussed at the beginning of the next chapter

The history of proliferation and regression in the mammae of any single species cannot be taken as typical of all differences are primarily not of kind but of the relative importance of the various phases of growth which are customarily recognized as contributing to full development and function phases dependent upon and owing their variable nature to the still more fundamental and characteristic reproductive history of the species It may however be less confusing bearing this proviso in mind first to consider mammary growth as it can be observed throughout the normal life history of a single species deferring a consideration of such points as are of interest in a comparative sense until the basic hormonal mechanisms have been discussed

The rat presents a species suitable for illustration in that it has been investigated in greater detail than most other mammals and also since it exemplifies a sufficiently representative selection of the possible growth phases associated with mammary development in other species The main details of its postnatal sexual life may be divided into (*1*) a period of inactivity lasting for about seventy days from birth to puberty (79) (*2*) periods of estrous activity in the virgin animal when an estrus cycle is evinced with a mean length variously computed at between 4 6 and 6 2 days (*120*), (*3*) *periods of gestation—approximately 22 days—* and of ensuing lactation when apart from a single estrus occurring one *day after parturition estrous manifestations are in abeyance for* about thirty days and (*4*) periods of resumed estrous activity in the post *parturient adult whose litter has been weaned* a process normally taking place when the young are about 21 days old Finally sterile copulation mechanical stimulation of the cervix and other agencies can induce a pseudopregnant period in the rat lasting for twelve to thirteen days

We may note here that the figure illustrating this section (Fig 1) depicts the rabbit rather than the rat gland this figure has been used so that readier comparisons may be made with the experimental glands of rabbits shown later in the text

Myers in a series of communications (103 105) has made an intensive study of the changes observed in the mammary glands of the female and of the male albino rat from birth to puberty His observations show that at birth the female glands have a mammary apparatus comprising second

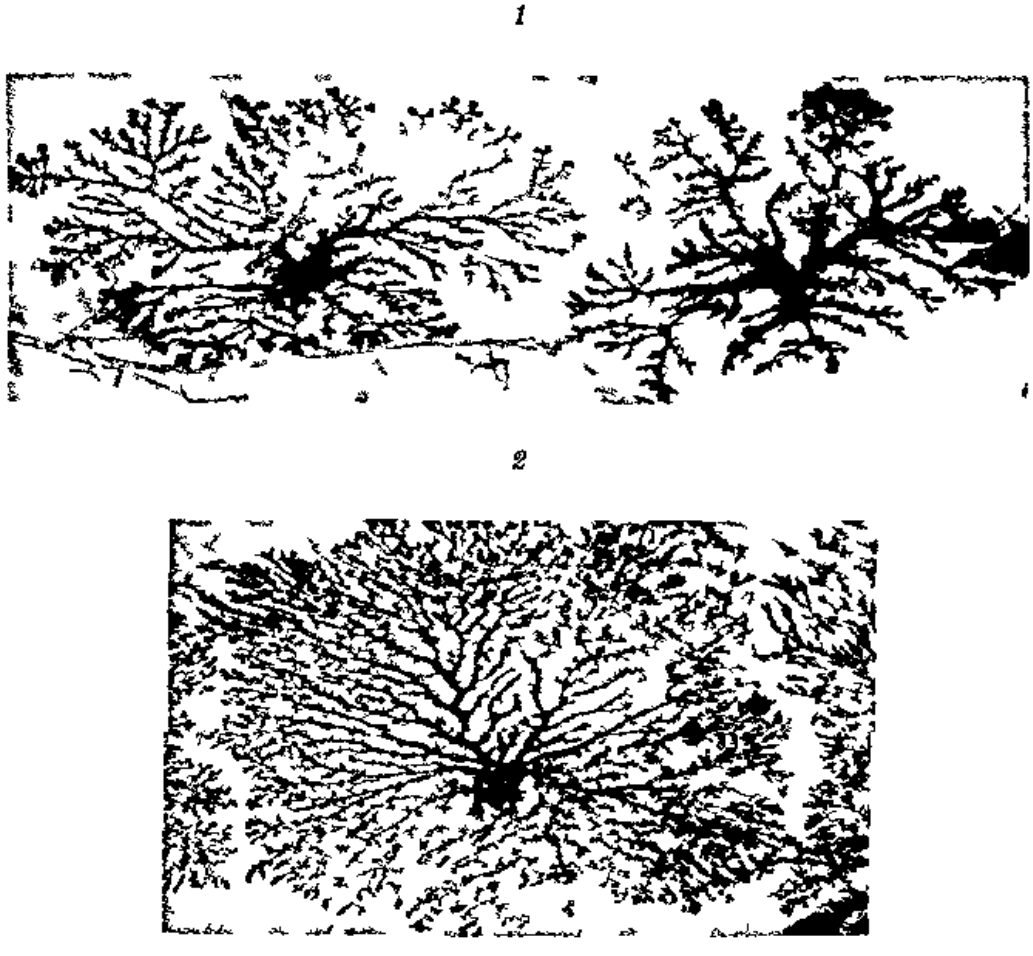
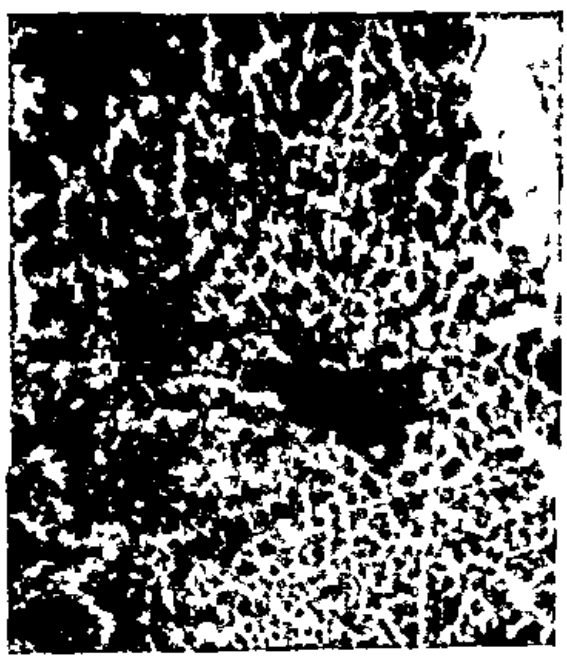


FIG 1 —Development of the female rabbit mammary gland (× 1) *1* Prepubertal *2* During first estrus *3* From an ovar ectomized adult receiving estro

ary, tertiary, and terminal ducts, all with lumina, as well as the partially closed primary duct The ducts ramify in a single plane parallel to the surface of the skin, except in the case of the second inguinal gland, where obstruction by the hind limbs or the external genital organs even at this



4



5

gen injections *4*. On twelfth day of pseudopregnancy *5* On twenty-third day of normal pregnancy. (From Parkes, 121,122)

early stage compels a multiplanar development Lateral and terminal buds are also apparent Allowing for a marked variation in the degree of development in different individuals, it was clear that the branching and extension of this immediately postnatal duct system proceeded only slowly for the first four weeks, an interesting change during this period, however, was the formation at two weeks of a continuous, but still very minute channel between the primary duct—and by implication the whole duct system—and the exterior Development, still by duct extension is far more rapid during the fifth week when separate gland systems begin to overlap, while in all glands the growth is no longer confined to one plane only A second phase of greatly increased growth takes place at the ninth and tenth weeks, and, whereas the earlier sudden proliferative burst was not connected by Myers with any concomitant physiological stimulus, this later enhanced activity could be ascribed with confidence to the onset of puberty At no stage during the prepubertal changes were true alveoli found and we may regard this period as one of extensive duct formation only, though "outpouchings" from the smallest ducts were deemed an early indication of lobulation

Further growth changes are imposed upon the duct system at each recurrent estrous cycle (149), when the main evidence is a rapid formation and sprouting of lateral buds on the smaller ducts This activity is followed however, by regressive changes during the next pro-estrous period and the net growth over the complete cycle is probably small and of very minor importance only as regards alveolar development

The formation of this secretory tissue is however abundantly evident during the first half of pregnancy Weichert and Boyd (170) found conspicuous lobule development in rats killed on or after the ninth day following conception but noted also that there was little further increase in the absolute amount of secretory tissue during the second half of the gestation period though the onset of alveolar secretory activity toward the end of pregnancy, with attendant distension of the alveolar lumina, resulted in an apparent extension of the parenchyma at this time Roberts (135), in an earlier study on mammary changes in the rat during the second half of pregnancy, had reached similar conclusions, stating that there was little further development during this period, in which the glands when sectioned resembled "small islands of glandular tissue in a lake of fat" Cytologically he found no evidence of mitosis in the alveoli during the period studied and concluded that the gland had "already reached its maximum growth, so far as number of cells is concerned, by the thirteenth day of pregnancy" From this time until parturition the changes are those associated with the hypertrophy of the formed glandular elements preparatory to active secretion, and with the first incidence of secretion

itself The cytological study by Weatherford (168) showed agreement with the contention that mitotic activity has practically ceased in the alveoli midway through pregnancy, but Jeffers (60), contrary to this view, claimed that mitotic figures are frequent during the second half of pregnancy and that hyperplasia therefore continues throughout this time

Maeder (90) has shown that the histological picture remains constant throughout lactation, and is the same in all respects as that seen in the incipient secretory phase at the end of pregnancy After weaning however, definite changes were noted by this author on the third day, and the involution of the parenchyma continued progressively from this time until the thirteenth day when the gland was judged to resemble closely that of the adult virginal resting gland, once again being composed only of ducts These observations substantiated an earlier and less detailed report by Myers and Myers (102)

The appearance of lobule-alveolar tissue is also to be seen in pseudopregnancy resulting from sterile copulation or mechanical stimulation of the cervix, and in the opinion of Freyer and Evans (31) the greatest proliferation during this time is qualitatively similar to that on the eleventh day of a normal pregnancy, though they judged the quantitative significance to be somewhat less Selye and McKeown (142) have reported that they too have never failed to detect alveolar development in rats pseudopregnant as the result of the suckling stimulus provided by litters from other does (143), and Jeffers (61) has supplemented their observations by a cytological study of the mammary glands of rats used in such experiments, expressing her opinion that the glands could have supported lactation comparable to that occurring after the birth of a normal, full term litter

A study of the male rat gland from birth to ten weeks of age (104) has shown a development parallel with that of the female gland for the first five weeks, after this, growth is much slower but may still continue to some extent even after puberty In contradistinction to most other species, both the extension of the duct system and alveolar development have been observed in mature males (4,88,165), the adult gland having a dense, compact development, while it is interesting to note the absence of nipples and the rudimentary condition of the second inguinal glands in all males examined

II Early Investigations

The earliest inquiries directed toward the elucidation of the mechanisms controlling the growth of the mammary gland have been adequately appraised in earlier reports (154,159), and we note here only the general

progress of thought in this field prior to the more modern work utilizing pure hormones or purified extracts with clearly defined endocrine properties

The original, and somewhat intuitive conception of neural control was overthrown at the turn of the century by experiments involving nerve section and transplants of mammary tissue (see Turner, 154, for review) The alternative possibility of hormonal mediation gained ground rapidly and was upheld as a reasonable physiological hypothesis by the growing appreciation of similar work in other fields, dating from the classic experiments of Berthold, Brown-Séquard, and others in the nineteenth century (see Chapter I of this volume) The close association of mammary growth with pregnancy naturally focused the attention of workers upon the fetus, placenta, and ovaries as the probable active agencies of control, and ovariectomies and ovarian grafts gave encouraging and provocative results Aqueous extracts of organs associated with the animal s reproductive life, for reasons easily surmized, were disappointing in their action—see for example the pioneering studies of Lane-Claypon and Starling (62)—and further advance awaited the first use in 1912 of lipide extracts (57) Progress remained comparatively slow, however, owing to the indefinite character of the extracts used, which did not permit satisfactory comparisons, either quantitative or qualitative, of one experiment with another The development of an easy assay of estrogenic potency by the Allen and Doisy vaginal smear test, following the indicative experiments of Stockard and Papanicolaou, and the subsequent isolation and characterization of the estrogenic hormones, quickly brought a greater objectivity into this realm of accumulating, yet unassessable, data, and we may regard these two achievements as demarcating the years of modern inductive work upon which our newer knowledge of the role of estrogens in mammary growth is founded The progress made in the years between these separate advances resulted from the use of ovarian, placental, and particularly urine extracts (52,64,159) of tested estrogenic potencies Such preparations gave the first clear indication of the relationship between the power to develop the mammary gland and the estrogen content of the active substances used and emphasized the need for pure ovarian hormone preparations as an essential step in the furtherance of research on the development of the gland

Apart from the ovary as a whole, the participation of the corpus luteum in mammary growth had been suspected from an early stage The proliferation of alveoli in pregnancy, in pseudopregnancy, and, in some species such as the dog, during metestrus could be correlated with the presence of active corpora lutea in the ovaries, but again the experi-

mental approach to the problem was hampered by inadequately characterized materials, until the preparation in 1929 by Corner and Allen of active corpus luteum extracts, and the isolation two years later of crystalline progesterone

The role of the anterior pituitary, now believed to influence fundamentally the development of mammary tissue, has been the subject of more recent research and its "history" is still that of contemporary endeavor and will fall naturally into the scope of this review as will also the more scant knowledge we have of the involvement of the thyroid and other endocrine glands

III. The Ovarian Hormones and Mammary Growth

A comparison of the growth caused by the purified estrogenic hormones, estrone and estriol, and by a crude extract of estrogenic hormones from pregnant cows' urine (161) showed no significant differences in the amount or type of growth produced The effects of these preparations were studied with the aid of three species rabbit, rat, and mouse and in each case it was clear that duct growth alone was being influenced and that the active principles in the urine extract were biologically similar to, and possibly identical with, the pure estrogens used in the experiment

The animals used in these and in similar studies having the same aim, were of both sexes When using females it is of course obligatory, in attempting experimental growth, to use animals spayed prior to puberty so that normal duct growth shall have been inhibited by deprivation of the natural ovarian estrogen supply, alternatively, in species such as the rabbit which show no appreciable development of the glands in the prepubertal stage (159), the use of immature unspayed females should be possible within this limited period On the other hand males of some species—since the male gland responds to the same stimuli and apart from the male ruminants (26), is equipotential with the female gland, while not being under the direct influence of the female gonad—frequently present more suitable experimental subjects, this is especially so in such cases as the mouse in which the male gland remains a mere rudimentary duct system throughout the normal life cycle (162) Castration has been shown unnecessary in several species, normal unoperated adults providing the best experimental subjects

An extension of the work on duct growth, usually with estrone as the estrogenic hormone, confirmed for all species studied the basic importance of these substances for this particular type of development, a uniformity contrasting markedly with the species differences encountered in the ability, or inability, of estrogens to influence the formation of lobule-alveolar tissue Concise summaries of the work bearing on both these

aspects of estrogen activity have been given by Nelson (107), Turner (156), Folley (21), and Petersen (124), and of laboratory animals, it will be sufficient to note in any detail here two of the most extreme instances the mouse, for which a pure duct response has been postulated, and the guinea pig, which by contrast gives complete mammary development after estrogen treatment

Turner and Gomez (163) demonstrated that 10 rat units (R U) of estrone administered daily to male mice would cause progressive growth of the duct system only, and that this growth was still to be seen after 100 days treatment, they concluded that the normal response of this animal would seem to exclude any lobule-alveolar formation The results were confirmed by Gardner *et al* (36), whose male mice, on treatment with estrone, developed glands comparable in size to those of the virgin female animal Even in this species, however, occasional reports (74) testify to the sporadic appearance of small isolated groups of alveoli in individual cases, though true lobule formation has not been observed as the result of physiological doses of estrogen in normal strains An abnormal development in which the duct growth was stunted, but lobules of alveoli were formed, was reported by Gardner *et al* (38) as the result of long-continued injections of estrone into both cancer-susceptible and cancer resistant strains, but the growth was admittedly atypical, even so the appearance of an alveolar epithelium, which was in some areas showing secretory powers as the result of simple estrogen treatment, cannot readily be discounted in any estimation of the role of estrogens in the development of mammary tissue in this species Similar results were obtained by Bonser (6), while evidence was adduced by Burrows (8), who painted the skins of male mice with different estrogens, that small differences in chemical structure could lead to appreciable differences in the biological responses evoked This investigator detected alveolar growth in a proportion of cases on all treatments, but noted particularly that estrone seemed to be mainly active in extending the duct system, whereas estradiol gave far greater alveolar development and had only a relatively slight effect on the ducts

More uniform results have been given by work on the guinea pig, and following reports by earlier workers (52 63) claiming definite lobule-alveolar augmentation and development equal to that seen in pregnancy in ovariectomized females and in normal males as a direct result of estrogen treatment, Turner and Gomez (163) found that 20 R U of their pregnant cows urine preparation injected daily for 40 days into adult males caused a glandular response similar to that seen in a female pregnant for 33 days Even injections of only 1 R U over a similar period gave clear indication of alveolar proliferation Similar results were

obtained with females which had been spayed before puberty These results were further supplemented by the work of Nelson (108), who, like Laqueur and co-workers (63), was able to report complete development of the gland judged by the spontaneous induction of lactation when treatment was curtailed, excellent corroboration was afforded by his histological studies

Of the other animals investigated it is thought that the normal response of the rat and the rabbit may be deemed primarily one of duct growth, with a slight degree of lobule proliferation in some cases (160 165), while simple duct extension is postulated for the cat and dog (158,164) As instanced in the case of the mouse however complete agreement among workers has rarely been achieved for any species and, while some small part of the disagreement may well be ascribed to differences of dosage, of the chemical nature or purity of the estrogen preparations used, or the varying age of the experimental animals at the time of treatment, a survey of the literature suggests the participation of intrinsic factors, to be considered in due course in this review and of which our present knowledge is most inadequate

First, however, and in further agreement with the instances of equivocal experiments already given we may note some conflict of opinion regarding the effects of estrogens on the mammary gland in primates In an undetailed report Turner and Allen (157) claimed lobule formation as the result of the long-continued treatment of a single normal male rhesus monkey This was confirmed by Gardner and Van Wagenen (39), who also obtained similar development following a shorter injection period, in the case of one spayed immature female A similar female given prolonged treatment developed glands resembling those of normal pregnant animals in type, though rather smaller in size Folley *et al* (22) on the other hand who also used the rhesus monkey obtained less uniform results, only one of thirteen male monkeys and two of four ovariectomized females showing any alveolar formation in response to estrone injections, in these the response was only slight and rendered less significant by the presence of a condition of gynecomastia with alveolar development, in one of a series of five normal untreated males which was also examined The work provided some grounds for believing the female gland of this species to be more responsive to estrogenic stimuli than the male It is perhaps of significance in assessing these various observations that the experimental periods chosen by Folley and co-workers were either much shorter (up to two months), or much longer (one year or more) than that found favorable for alveolar development in the male by Gardner and van Wagenen (six months) Gardner (33) has more recently reported complete morphological development in

young male and female monkeys given estradiol benzoate injections for periods of approximately eight months, and has also obtained an indication of an inhibitory effect on mammary growth when the weekly dosage exceeds 0 4 mg

The recent discovery (15) of synthetic products chemically distinct from but biologically similar in their action to the natural estrogens and the production of these substances both cheaply and in quantity have led to an immediate extension of the investigations involving estrogens to cover the major domestic animals of importance to the dairy industry Lewis and Turner (74,75) confirmed the power of diethylstilbestrol to act upon the mammary glands in very much the same way as do the natural estrogens when administered to mice, rats, rabbits and guinea pigs and it will be legitimate therefore for us to include studies with this and similar synthetic products in our consideration of estrogenic influence

de Fremery (29,30) had already reported mammary growth in the virgin goat following percutaneous inunction of the udder region with an ointment containing estradiol monobenzoate, and his results were confirmed by similar inunction experiments in which diethylstilbestrol was used as the active substance (25,26) Administration of this synthetic hormone by injection in oil, by implantation (Fig 2), or orally has also caused mammary growth sufficient to maintain abundant lactation in this species (74,77,78) It is of particular interest to observe the successful use of an ovariectomized female in the experiments conducted by Lewis and Turner (77), as also in the first encouraging report of similarly induced udder growth—inferred from the occurrence of lactation—in the bovine (167) The implication that progesterone at any rate from an ovarian source, is not required for full glandular development in these two species will be more fully considered in due course Confirmatory evidence of mammary growth in the bovine following treatment with synthetic estrogens has since been given by Reece (128), Folley and Malpress (23 24) Hammond and Day (50), and Parkes and Glover (123), among others In all these reports the evidence for lobular as well as duct growth cannot be open to doubt, both the resulting measure of lactation in some cases equalling that of normal parous animals, and the macroscopic appearance of whole udder slices bearing clear witness to this end In most cases, too, it seems likely that luteal influence was excluded since the ovaries were hypoplastic during treatment (23 50) A histological study by Mixner and Turner (101), however, has suggested that in virgin female goats the development may not always be truly normal following diethylstilbestrol treatment, and in any case both goats and cows have shown wide individual variations in response to estrogens, growth of udder tissue following the same treatment has

ranged from a complete absence of response to the formation of full sized glands In many cases it would seem that these two species offer a parallel to the complete mammary growth following estrogen stimulation which has been noted for the guinea pig a development that clearly cannot, without qualification, be explained on any simple theory whereby estrogens are held responsible for the promotion of duct growth alone It must however be remembered that diethylstilbestrol, on which our main conclusions for the cow and goat are based may differ from the

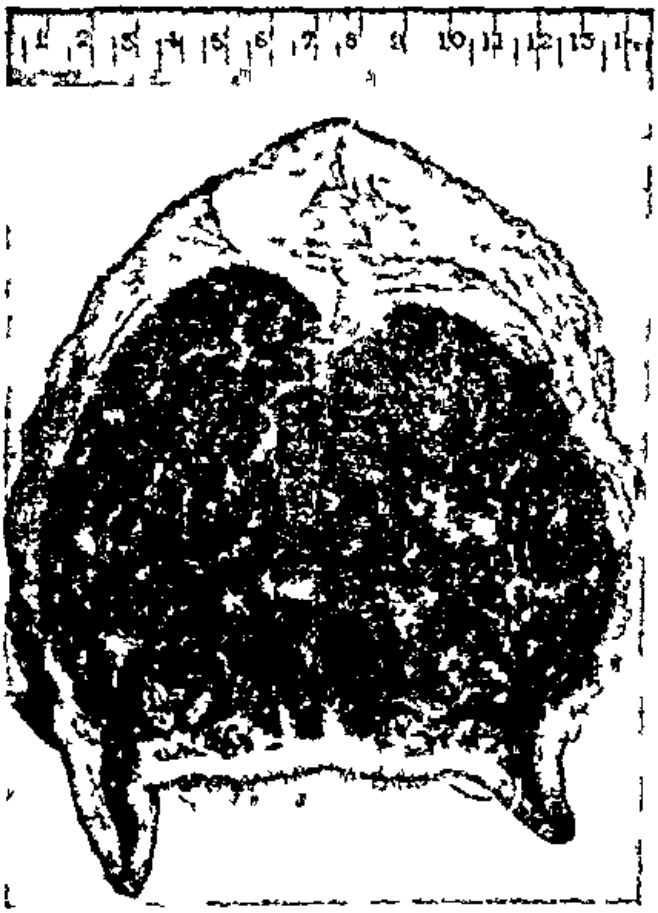


FIG 2 —Section through whole virgin goat udder showing glandular development following subcutaneous implantation of synthetic estrogen tablets (Scale in centimeters)

natural estrogens in the ability to proliferate lobular tissue (77), a hypothesis that receives support from the lobule formation observed by Mixner and Turner (101) as a result of diethylstilbestrol and estrone injections given to ovariectomized mice, and from the work of Burrows already quoted (8), but against which we may cite the mammary growth obtained by de Fremery (30) in the virgin goat given injections of estradiol benzoate

It will be useful at this point to consider experiments designed to

throw light on the role of progesterone in mammary tissue formation, before attempting to give any general conclusions on the relative importance and significance of the ovarian hormones in the parenchymatous development of the gland The complementary relationship existing between the ovarian hormones in their action upon the uterus, as evidenced by the proliferative and progestational phases of endometrial development is now deemed to have a near parallel in the associated phenomenon of mammary growth We have seen that only rarely does the fullest development of mammary tissue follow simple estrogen treatment in males or ovariectomized females, the guinea pig providing the only well attested case for which both alveolar and duct formation in similar amounts to that occurring in normal pregnancy can be regularly obtained The larger domestic animals seem to be subject to much individual vagary of response, a result which, were estrogens the only hormone needed for full mammary development, we should not *a priori* expect Attempts to develop alveolar growth by progesterone treatment alone, however, proved in vain in many species, and early optimistic reports by Nelson and Pfiffner (114), who claimed lobule development in male and spayed female guinea pigs, rabbits, and rats in response to injections of corpora lutea extracts, were later withdrawn (108) on suspicion that some estrogen had in fact been present in the relatively crude extracts used Turner and Schultze (165) injected lipide extracts of corpora lutea into castrated male and female rats and found no lobule or end bud formation results which were reproduced for the rabbit (160) and for the guinea pig (163), for the latter animal it was observed that the extract was ineffective even after a preliminary treatment with estrone An interesting yet anomalous result was that reported by Gardner and Hill (37) who found an extension of the duct system in male mice, both castrated and noncastrated, after injections of highly purified extracts of progesterone The presence of slight estrogen contamination, although unlikely in quantities sufficient to affect the result, was not absolutely excluded as a possibility This work has been extended by the observations of Mixner and Turner (98), who obtained lobule alveolar growth in castrated female mice by injecting high doses (3–7 mg) of progesterone alone Conflicting reports have been given by workers using the rat as an experimental animal and in the view of Selye (138) negative responses recorded earlier by himself and co workers (139), and by Astwood *et al* (4) may be ascribed to the insufficiently large dosages given In his later work (138) as also in that of Reece and Bivins (129), 15 mg progesterone given daily over a period of ten days to mature ovariectomized rats induced definite lobule alveolar development There is disturbing evidence, however, that the degree of proliferation might depend on the

time of ovariectomy relative to the time of starting treatment, decreasing as this postoperative period increases (138) This would imply a possible participation of residual unmetabolized and unexcreted estrogens in the positive responses Doses of a similar order given to mature ovariectomized female rhesus monkeys for periods of approximately one month, have also led to an increase in the amount of lobule tissue present in biopsy specimens (51) Further studies are clearly desired both to confirm these results and to extend the use of these comparatively high doses of the purified hormone to the investigation of the proliferative power of progesterone when given alone in other species

The occasional reference to the inability of a preliminary treatment with estrogens to prepare the gland for an active response to subsequent progesterone injections is in sharp contrast with the results obtained with simultaneous injections of these two hormones Here the reports have been most uniform attesting to an alveolar response resulting from the hormonal synergism superimposed on the customary estrogen duct stimulation Turner and co-workers (158 160 163,165), for the rabbit, rat, mouse, and cat, and Anselmino *et al* (2) and MacDonald (87) for the rabbit, have all helped to establish firmly this synergistic relationship which results in the formation of glands very similar indeed to those of normal pregnant animals, in the case of the guinea pig in which estrogen alone can give full lobule formation, the supplementary treatment with progesterone failed to alter the character of the tissue or to induce a more extensive development (108)

More recently attention has been directed to the proportions in which the two hormones have to be given in order to achieve an optimal result In particular, Lyons and McGinty (83), using male rabbits given a standard daily dose of 120 I U estrone studied the effect of daily doses of progesterone varying from 0 25 to 8 I U Synergism was maximal with 1 I U (*i e*, 1 mg), although not productive of the full development to be observed in pregnant animals (Fig 3) Further experiments were therefore performed in which the progesterone dose was kept constant at this optimal level, but the estrone dosage varied from 30–960 I U daily (137) The best results, although still not fully equivalent to the proliferation in the glands of pregnant animals two to three weeks after conception, were given by those groups receiving 240 and 960 I U , but, as male animals were used whose glands were of course initially more rudimentary than in the female, it is probable that the development did represent very closely the natural conditions of mammary development for this species (Fig 4) A similar study in which ovariectomized virgin female mice were used has been reported by Mixner and Turner (98) With a constant daily estrogen dose of 133 I U these authors observed a

satisfactory growth of lobules with 1 0 to 1 5 mg progesterone daily They further showed that, if progesterone administration was held at 1 mg daily, optimal synergism was given over an estrone range of 40–133 I U Unfortunately their range of estrone doses jumped from 133 to 1200 I U , at the higher value the lobular response, although suboptimal, was still evident It would seem therefore that the relative amounts of

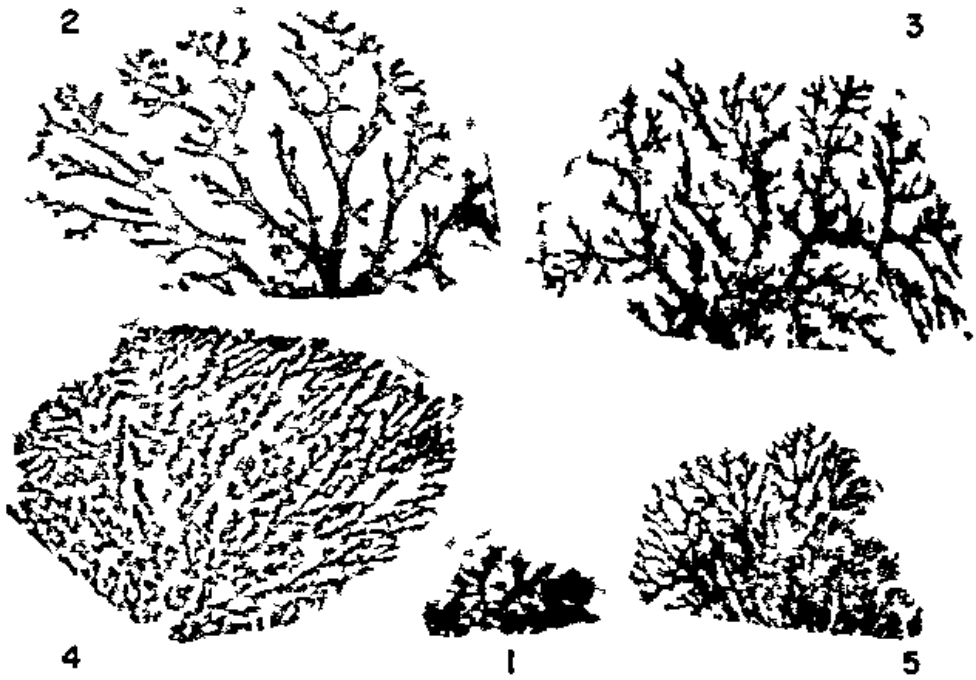


FIG 3 —Experimental development of the rabbit mammary gland Figures represent approximately one half of a male rabbit mammary spread after eighteen injections given over a 28-day period (× 1 5) Single injections 1 None 2 120 I U estrone 3 120 I U estrone and 0 25 I U progesterone 4 120 I U estrone and 1 I U progesterone 5 120 I U estrone and 8 I U progesterone (From Lyons and McGinty 83)

the two ovarian hormones required to evoke the fullest mammary response is of the same order for the mouse as for the rabbit Since the international unit of estrogens is equivalent to 0 1 μg estrone, we may regard the evidence from these two species as suggesting a proportional relationship of about 40 1 (progesterone estrone), by weight, for the best mammary development

It is of great interest, in view of the proportionality found necessary for effective synergism in mice and rabbits, to contrast the authors' unpublished and as yet incomplete results on the simultaneous implantation of goats with progesterone and estrogen tablets, with similar work, in which the hormones were injected, carried out by Mixner and Turner

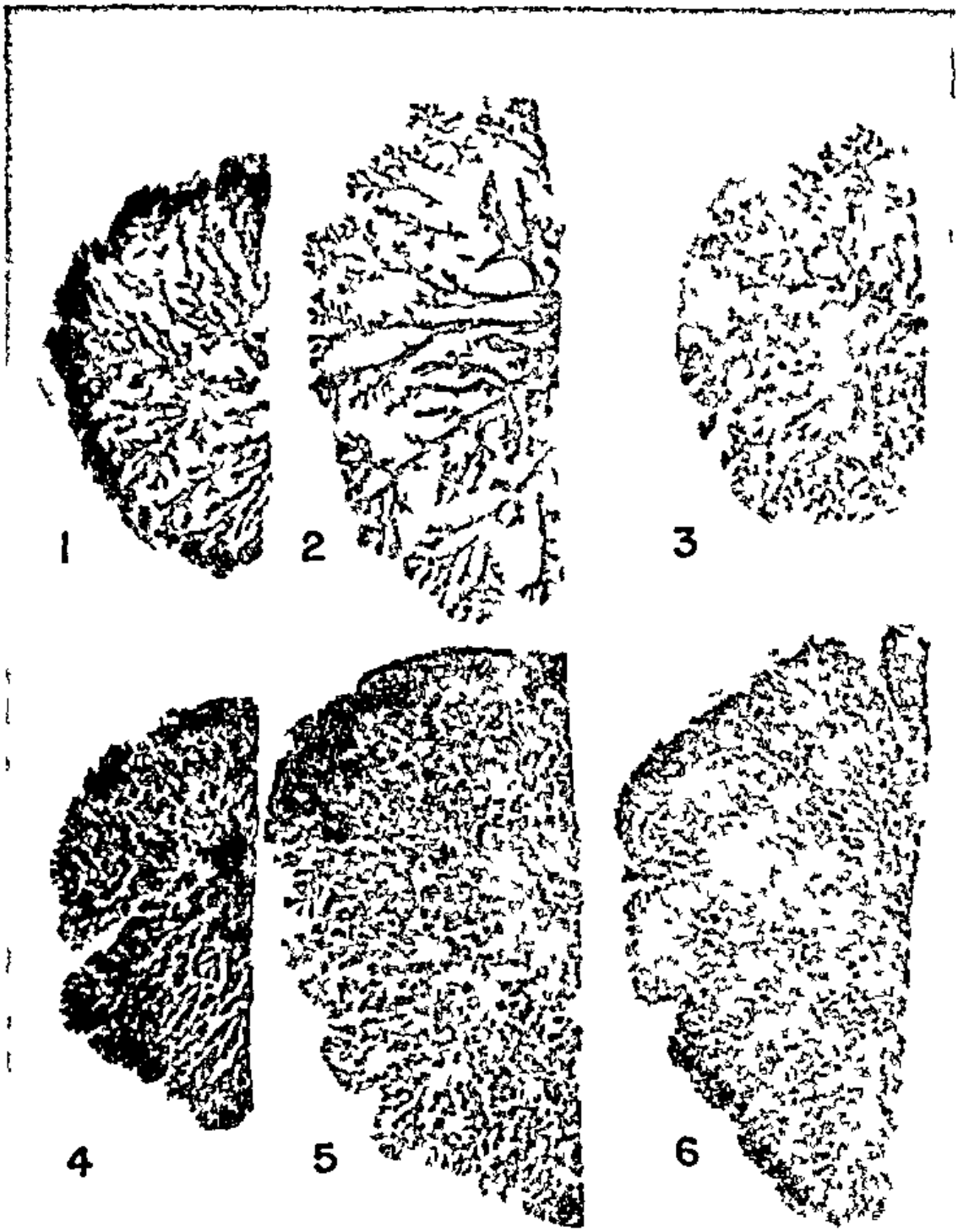


FIG 4.—Experimental development of the rabbit mammary gland Figures represent approximately one half of a male rabbit mammary spread after 25 injections given over a 35-day period (× 1 5) Single injections *1* 30 I U estrone *2* 240 I U estrone *3* 960 I U estrone *4* 30 I U estrone and 1 I U progesterone *5* 240 I U estrone and 1 I U progesterone *6* 960 I U estrone and 1 I U progesterone (From Scharf and Lyons 137)

(101) In the former experiments the gravimetric ratio of progesterone absorbed to estrogen absorbed was never in excess of 9 1, and more usually about 2 1 The estrogen used—hexestrol—has, like diethyl stilbestrol been variously computed to have a biological activity 2 5 times as great as (15), or equal to (19), that of estrone, and, although as shown by Emmens (18) too much reliance should not be placed in comparisons of one estrogen with another, since the relative activities may vary with the assay used or even the technique adopted, the possibility remains that the effective ratios were smaller still The highest mean daily absorption of progesterone for these animals was about 5 mg It is perhaps not surprising, therefore, that no evidence of increased alveolar development could be observed even after 100 days in those animals given the dual hormonal treatment, when compared with controls receiving the estrogen alone Mixner and Turner (101) however were able to report development after sixty days corresponding with that of midpregnancy in goats receiving 20 or 30 mg of progesterone daily, and diethylstilbestrol sufficient to give a ratio of progesterone diethylstilbestrol of 200 1 by weight The difficulty, when working with goats in such small experimental groups, of ensuring adequate controls, having in mind the very wide variations of response given after simple estrogen treatment, necessarily prevents any strict conclusions being drawn from these contrasted results, but they do suggest a possible uniformity in the quantitative synergism of the ovarian hormones in their effect on mammary growth in different species

Using the semisynthetic progestational hormone—pregneninolone or ethinyltestosterone—together with estrone, Mixner and Turner (96) have demonstrated a synergism for mammary growth in spayed female mice very similar indeed to that shown by the natural hormone itself There have recently been other reports too, indicating that progesterone is not even the only naturally occurring hormone which may develop the lobule-alveolar system In particular the involvement of the steroid adrenocortical hormones must be considered a real possibility, and further work in this field is urgently required A relationship between the adrenal cortex and the mammary gland had been inferred by some authors as the result of clinical observations on the association of cortical tumors in the male with gynecomastia, but no experimental studies were available until van Heuverswyn *et al* (54) obtained extensive duct growth in unoperated or castrated male mice, following the injection of 4 0 mg desoxycorticosterone acetate on alternate days over a period of sixteen days The response was greater than that given by estradiol benzoate given similarly in daily doses varying from 0 016 to 0 666 mg No lobule alveolar growth was recorded by these authors Nelson *et al*

(111) have reported mammary growth in young male guinea pigs after similar injections, and since lactation started in some cases when treatment was stopped we may reasonably infer that alveolar tissue had been formed Speert (146) also found active lobule-alveolar growth in the glands of two adult ovariectomized rhesus monkeys given daily 10 mg desoxycorticosterone acetate for 13 and 24 days Five mg given daily for 30 days failed to stimulate growth in a third monkey, however Mixner and Turner (99), comparing the power of various steroid substances to produce lobule alveolar growth in virgin ovariectomized mice given supplementary estrogen treatment, rated desoxycorticosterone acetate to be one third as active as progesterone, and also noted that acetoxypregnenolone—another related steroid—had approximately one sixteenth of progesterone s activity Reports on the effect of adrenalectomy on underfed (9) and normally fed (133) rats given sodium chloride therapy have agreed in part Thus increased growth compared with glands from control animals was observed insofar as the number of lateral and end buds were concerned, though the area of the glands was affected only in the first-mentioned experiments The mechanisms responsible for these changes are in doubt, though direct ovarian implication would seem unlikely since the same changes were seen in normal-adrenalectomized and spayed-adrenalectomized animals In a more recent investigation Cowie and Folley (13) have failed to confirm this proliferative effect of adrenalectomy in fact the mammary gland area was significantly decreased by the operation

Leaving aside any question of pituitary mammogenic activity, which according to modern theories depends on preliminary evocation by ovarian and possibly other related hormones, we may fairly assess control of mammary growth as originating in ovarian and adrenal relationships It will be evident that any attempt to form a concise theory of this development will be baffled by two recurrent observations first, the variation among species encountered in the experimental growth of the gland, ranging from the mouse, which apparently requires both estrogen and progesterone for the development of a potentially secretory parenchyma, to the guinea pig for which estrogen alone has been deemed sufficient, and second, the variation within species, for examples of which we may instance the alveolar development following estrogen treatment in certain strains of mice, and the very wide differences in the mammary response of heifers and cows given simple exogenous estrogen applications On the other hand, certain facts regarding the hormonal regulation seem to be cardinal for almost all the mammals which have been relatively intensively studied Chief among these we may note the ability of estrogens to cause, at least, an extensive duct growth—though

the inability hitherto to increase in any marked degree the gland of the male goat by such treatment must be recorded as an exception (30,77)—and the probable power of estrogen and progesterone, provided they are administered simultaneously and in the correct proportions, to ensure a *full mammary development* in normal or ovariectomized females, as also in the males of most species investigated Here again we must cite the evidence that the guinea pig gland can proliferate fully without any intervention of progesterone at all We are therefore posed with the question do true species variations exist in the qualitative hormonal influences required to form the mammae of different animals—a theory which may most easily explain observed experimental data, but which on purely *a priori* grounds might well be unconvincing—or might there be an alternative hypothesis whereby common hormonal factors are held responsible for duct and alveolar growth, respectively, whatever the species and a concordancy of the established results is sought on the basis of quantitative rather than qualitative differences?

The arguments in favor of the first view may be fully appreciated by any direct reading of the experimental inconsistencies to be observed in reports relating to different species and it is the possible alternative that we shall consider here

A warning was first sounded by Folley (21), who pointed out that, since the isolation of progesterone from a concentrate of ox adrenal glands by Beall and Reichstein (5), none of the experiments purporting to have obtained alveolar development in males and immature or ovariectomized females as the result of estrogen treatment alone could in fact be interpreted with certainty in this way The realization that at least one of the adrenocortical hormones—desoxycorticosterone—also has very pronounced progesterone like properties in its action on the mammary gland has further focused attention on the adrenal cortex as an alternative source of hormones stimulating lobule-alveolar proliferation It is interesting too to note the excretion of pregnanediol by rabbits after injections of desoxycorticosterone acetate (55), and the later report of a similar conversion in man and the chimpanzee (56) The intermediates in this change remain hypothetical but it is not impossible that some of them might also possess and be of sufficient permanence in the tissues to exert progestational powers Similar biologically active substances could be envisaged as arising in the anabolism or catabolism of the other steroid cortical hormones, our knowledge of steroid metabolism is, however so small at the present time that we cannot with any confidence assume that the metabolic pathways are the same for all species, or even if this should be so that the rates of conversion and destruction have any species uniformity Reports of the progestational activities of adrenal

extracts from horses, cattle, and pigs have been recorded (10,20) It will be readily appreciated, therefore, that progesterone itself can no longer be considered a specific hormone for the mediation of alveolar growth, even though the evidence is still strongly in favor of its premier role in this respect but that the adrenal gland must be henceforth regarded as having supplementary powers in this function This conception immediately suggests a possible explanation for the interspecies differences found in experimental studies since quantitative variations alone in the amount of progesterone like substances produced by the adrenal cortex in different species could presumably condition widely divergent mammary responses to simple estrogen treatments Further studies on the metabolism of the adrenal steroids and on the biological properties of intermediary substances especially if undertaken with a view to the comparative biochemistry of these changes should greatly clarify the confused picture which has until recently been presented by the hypothesis of simple ovarian control of mammary hyperplasia and might possibly establish a complete absence of lobule-alveolar activity on the part of estrogens for all species These considerations will of course be unaffected by any conception of pituitary participation in mammary growth provided such mediation is itself under ovarian control

Since it would seem from various studies already quoted that the estrogen progesterone like substance ratio has to fall within certain defined limits for optimal synergism the path and rate of metabolism of estrogens, and of their excretion might also account for response variations among species Here again our present knowledge of metabolic changes is far from complete though recent work would seem to show that the pathways and excreted products may well vary among species (150), and also within species depending on the presence or absence of other hormones, amount of estrogenic hormone present, and other fluctuating criteria (see reviews by Doisy *et al*, 16 and Pincus and Pearlman, 126, for admirable and recent summaries) We can therefore carry our argument one stage further, to afford a reasonable explanation of the intraspecies variations obtained by different workers using different strains of the same laboratory animals, or by all workers in their own experiments when using the larger domestic mammals

For, where two factors, the metabolism of both the endogenous and administered hormones in their qualitative as well as quantitative aspects, are unknown, and the potential supplementary endogenous progestational activity of the adrenal gland is a matter of pure conjecture, uniformity of response will probably not readily be obtained by uniformity of treatment, it is perhaps in accord with this view that

undoubtedly the largest intraspecies variations so far recorded have been those found following estrogenic treatment of the larger mammals—cows and goats—whose widely different potentialities under normal conditions are frequently suspected, at least in part, to be hormonal in origin The prolonged hypoplastic condition of the bovine ovary under estrogen stimulation (23) will of course make extraovarian sources of progesterone of supreme importance, and despite the presence of ovaries in the treated animals a condition tantamount to ovariectomy is in fact imposed

IV The Anterior Pituitary Gland and Mammary Growth

Probably the most lively interest at the present time in connection with the endocrine control of the mammary gland revolves round the role of the pituitary and the controversial question of the existence of specific mammogens secreted by this gland It would be premature in the present state of our knowledge to attempt any definite statement, but we may recognize three distinct viewpoints in favor of which evidence exists, and for which further confirmation is still being sought These are, first, the mammogenic theory propounded by Turner and collaborators (73,101), which claims that the ovarian hormones, although essential for the growth of the gland, do not exert their effects directly, but indirectly through the mediation of the anterior pituitary gland It is held that the pituitary, following stimulation by estrogens and progesterone, secretes specific "mammogens" of its own, which are in fact the hormones acting directly on the undeveloped mammary structures Two mammogens are postulated, one evoked by estrogen stimulation and known as the "duct growth factor," the other by progesterone and related substances, or estrogen and progesterone together and known as the "lobule-alveolar growth factor" It will be clear that this modern hypothesis, if accepted, will in no way render invalid the earlier work on the mammary function of the ovarian hormones, but that these substances will retain their fundamental importance as primary agents of growth Secondly there is the more conservative attitude which holds that those who support the mammogenic theory have failed to sustain their claims by clear cut experiment, and that all effects so far observed are explicable on the basis of direct ovarian control, and lastly there is the intermediate view (131), more recently expressed, which attributes growth to a synergistic relationship between the ovarian hormones and pituitary mammogens

It may be that species differences, age differences, and like factors may here again be basically responsible for much of the disagreement among the results of separate groups of workers Recent experiments on the effects of steroids on the mammary glands of hypophysectomized

rats by Leonard (67) and Smithcors and Leonard (145) may be interpreted as indicating that the age of the experimental animal, both absolute and relative to the time of hypophysectomy, may greatly affect the results obtained, but it seems more likely that confusion arises from problems of experimental technique and in particular the fact that most of the critical experiments to test the involvement of the pituitary necessarily demand the use of hypophysectomized animals In such work the danger of incomplete hypophysectomy presents a very real difficulty Gomez *et al* (46) have shown that, in the presence of residual fragments amounting to such a small total as 2% of the excised gland, the mammary response of hypophysectomized male mice following estrogen treatment could be changed from a negative to a positive response essentially the same as that found in intact animals similarly treated Substantially the same results have been reported by Gomez and Turner (44) for the guinea pig, rat, rabbit, cat, and ground squirrel The authors in consequence stressed the danger of interpreting positive responses in hypophysectomized animals treated with estrogens as evidence of an absence of pituitary mediation in mammary growth changes, unless a rigorous postmortem histological examination of the *sellae turcicae* of the experimental subjects had established with certainty the thoroughness of the operation

Further, it is well known that hypophysectomy necessarily involves other changes in the experimental animal affecting its general well-being and its fundamental endocrine relationships, so rendering it in many respects abnormal As a result of this, and in contradistinction to the attempts to attribute the positive responses of hypophysectomized animals following estrogen treatment to incomplete hypophysectomy, some workers have adduced evidence that, in completely hypophysectomized animals, negative responses to estrogen may be due to general depressive effects of the operation, such as a lowering of the plane of nutrition Astwood *et al* (4) tested this possibility by injecting two groups of intact young rats with estrone for a period of fourteen days, one group was placed on a restricted diet, approximating that consumed by hypophysectomized animals, so that weight was lost throughout the experimental period, while the other group was fed normally and gained weight The latter group showed a typical extension of the duct tree following treatment, comparable growth was not seen in the case of the poorly fed animals The authors suggested therefore that the arrest of body growth following hypophysectomy might explain the observed failure of estrogen to prevent the regression of the mammary gland encountered under these conditions in the immature rat Nathanson *et al* (106), developing this theory, found that hypophysectomized rats

treated postoperatively and simultaneously with estradiol benzoate and a pituitary growth complex preparation which was itself inactive mammogenically, showed graded mammary responses which directly paralleled the weight increases following the operation, but, since, even so, the degree of development never approached that given by intact animals treated with estrogen alone, they were inclined to assign some mammogenic activity to the hypophysis itself, despite the clear "nutritional" effect Samuels *et al* (136), on the other hand, in experiments in which the weight of hypophysectomized rats was maintained, not by injections of growth hormone but by forced feeding coupled with desiccated thyroid administration, failed to demonstrate any such connection between the plane of nutrition and mammary development, they concluded that Nathanson *et al* had achieved their results solely by virtue of some factor, present in the pituitary extract they used, having either mammogenic activity or an essential metabolic function evinced only in the presence of estrogens Trentin and Turner (153) have observed an inverse relationship between the food intake level of normal male mice and the amount of estrogen required to produce a minimum duct growth response They interpret this, in the light of other known depressive effects of inanition on the secretory activity of the pituitary, as indicating pituitary participation in ovarian mammary relationships Their contention, however that, if the action of estrogen on the mammary gland were direct the response would, if anything be increased by inanition, is based on the imperfect analogy that inanition may lead to a heightened sensitivity of certain pituitary controlled glands to hormonal stimuli, and must be regarded as most dubious

Finally attempts at replacement therapy by different pituitary fractions have all too frequently involved difficulties of interpretation and comparison owing to the variable or even uncertain composition of the extracts used, and the crippling doubts in any case regarding the reality of their mammogenic potencies

It will be clear therefore that attempts to solve the riddle of pituitary mammogenic function are fraught with difficulty and rarely permit any conclusions which can be accepted without some reservations In particular, the imperative need for checking the thoroughness of pituitary removal in experiments involving hypophysectomy may be deemed to throw a cloud of suspicion over much of the early work in this field, which remains one of speculation for the scientist and will probably require some additional, new experimental approach before its enigmas are fully solved

The conflicting evidence accumulated from the attempts to determine the action of ovarian hormones, particularly estrogens, on the mammary

glands of hypophysectomized animals, and the effects of pituitary implants and extracts on normal and hypophysectomized animals has been ably reviewed by several authors (21,107,156) More recent reports in which these methods have been used have failed to clarify the problem as may be seen by reference, for example, to the work of Reece and Leonard (131), who were unable to stimulate any mammary development in hypophysectomized rats treated with estrogen alone and to the impressive study of Fredrikson (28), who using hypophysectomized rabbits found that treatment with estradiol monobenzoate and progesterone caused just as much glandular development, both of ducts and alveoli, as could be expected in normal rabbits similarly treated thus fully confirming the earlier work of Asdell and Seidenstein (3) on this species

An important step in the history of this investigation was taken when Gomez et al (47) reported that, if male hypophysectomized guinea pigs were each given an implant of one male rat pituitary daily for twenty days, extensive alveolar development could be produced comparable with that given by injections of estrone into normal guinea pigs, provided only that the pituitary implants were obtained from rats previously injected themselves with estrogen This work, apparently providing positive evidence of a pituitary factor essential for mammary development and formed as a result of estrogen stimulation, provided the germ of the mammogenic theory The authors distinguished it from the lactogenic, thyrotrophic, and adrenotrophic hormones, preparations of which, under similar conditions, had failed to give any commensurate mammary response (44) Confirmatory evidence was produced (45) when duct and alveolar growth were stimulated in immature spayed female rabbits and rats by injections of fresh pituitary material obtained from cattle in the first half of pregnancy Similar injections of pituitary powder from nonpregnant heifers failed to stimulate such growth, again there seemed therefore to be an interdependence between the production of "mammogens" and the ovarian hormones Since the latter experiments were conducted using ovariectomized animals as the test subjects, a tentative conclusion could also be drawn that the action of mammogens on the mammary gland was unaided and did not involve any synergistic relationship with ovarian steroids However, these results have not been unfailingly reproduced by other workers, Nelson (109), for instance, using hypophysectomized female rats and adult male mice obtained the same mammary growth following implantation of pituitaries from untreated rat donors, as he did from those of estrogen pretreated animals, while Reece and Leonard in similar experiments (130), also reported the same degree of development in both cases The latter authors supported the claim for the existence of a specific hypophyseal mammogenic hor-

mone on the grounds that their implanted animals always showed a greater mammary development than unimplanted hypophysectomized controls

Seeking to develop their mammogen theory, Turner and colleagues formulated an assay technique for their duct growth factor—mammogen I (73) Normal male albino mice were found to give a suitable biological response to subcutaneous injections of fresh macerated anterior pituitary tissue taken from pregnant cattle, involving the appearance of thick ducts with side branches and large club-like end buds On the basis of these observations a mammogenic mouse unit was defined as the amount of tissue or extract, given subcutaneously once daily for six successive days, which would produce definite signs of development in one or more glands of 50 ± 10% of a minimum of ten male albino mice weighing 15–25 g , the glands being removed on the seventh day In its present form, however, the absence of a clear increase in the percentage of positive responses attendant upon an increase in dosage must be regarded as an unsatisfactory feature of the test, and it seems inadequate to explain a decreased response at higher levels by reference to the established and supposedly parallel stunting effect of large amounts of estrogens on the mammary gland In the latter case we are considering an over dosage beyond the limit of positive biological response, in the former the apparent inability to reach even a threshold value in the case of about 40% of the mice involved in a given test It is, in any case, confusing to compare the varying effect that an increasing dose of a substance may have on individual animals with the fluctuations in the percentage evocation of response in a group of animals The possibility of refractoriness in some of the mice seems more plausible, or indeed that endogenous factors are influencing the assay to a variable extent in different individuals With the aid of the assay method an increase in the mammogen I content of cattle hypophyses during the first half of pregnancy was demonstrated, and values were also obtained for the hypophyses of nonpregnant cattle, for pregnant rabbits, and male rabbits pretreated with varying amounts of estrone Since in many cases, however, the stringent conditions of the assay appear to have been disregarded and only the merest approximations to a correct value obtained—deduced questionably from responses falling outside the 50 ± 10% range, and from test groups of less than ten mice—further work will be required before the findings can be accepted without reserve

Attempts to identify the mammogen duct growth factor with any of the known pituitary principles have met with little success Interest has mainly centered around possible associations with the growth hormone or lactogenic fractions, and the results of Nathanson *et al* (106)

already quoted might be construed as evidence in favor of participation of the growth hormone in mammogenic responses in synergism with estrogens Reece and Leonard (131) also found that for the hypophysectomized rat the growth hormone preparation used seemed to supply the necessary substance enabling the mammary glands to respond to estrogen treatment Gardner and White (40) on the other hand have demonstrated mammary growth in hypophysectomized male mice following the simultaneous injection of estrogen and purified prolactin preparations, and Gomez (42) also was able to report extensive duct growth in hypophysectomized castrated guinea pigs provided the treatment with prolactin and estrogen was sufficiently prolonged and the dose of pituitary principle sufficiently high He was inclined however to attribute the response to distinct mammogenic factors present in his prolactin preparation Supplementary data accrued from this and other work in which pituitary extracts were used showing that the significant amounts of other pituitary hormones frequently present were ineffective in causing any mammary proliferation Gardner and White (41) however, using hypophysectomized male mice reported contrary to most other workers some mammogenic activity resulting from concurrent injections of estrogen and a pituitary extract having marked adrenocorticotrophic activity The "lactational growth observed by Lyons (80) and restricted to those sectors of proliferated lobules which he directly injected with lactogenic hormone preparations has been more fully considered in the next chapter but should be noted in the present context

The claim that the pituitary duct growth factor, unlike other hormones from this gland is soluble in fat solvents (72 73), has more recently been reinvestigated It is now believed (151) that the factor does indeed resemble other pituitary principles in that the activity after extraction of the fresh glands resides in the protein fraction The significance of much work published on the mammogenic properties of lipide extracts of pituitaries is now therefore rendered of small account Greep and Staveley (48) first drew attention to the error of attributing duct growth powers to lipide extracts when, using such solutions obtained from cattle pituitaries which were themselves able to induce duct growth and end bud formation in spayed and hypophysectomized immature female rats, they found that the mammogenic activity of the original whole tissue had not been extracted, but in great part was still present in the tissue residues

It will be appreciated that, in view of the discrepancies and general lack of agreement bearing on fundamental aspects of the mammogen theory so far as it relates to the duct growth factor, any premature

dogmatism regarding its postulates must be dangerous It remains a pressing field for further research particularly for the revision of assay techniques and the better characterization or isolation of the active principle

The literature concerning mammogen II, the lobule-alveolar growth factor, has been reviewed by Mixner and Turner (101) A preliminary report (93) demonstrated the ability of injections of fresh anterior pituitary material from cattle to cause lobule-alveolar growth in young ovariectomized virgin female mice Development akin to that seen in pseudopregnancy was obtained in some cases A tentative but unsatisfactory assay technique was developed using spayed virgin mice as the test animals (94) They were injected under specified conditions with the pituitary material under assay, but the alveolar responses were irregular and the method was superseded by one in which the pituitary injections were given with simultaneous estrogen administration In its final form (101) the test still makes use of the nulliparous spayed mouse weighing between 12 and 18 g as assay animal, which is injected subcutaneously once daily for ten days with the material on test and with 7 5 I U of estrone Glands are removed on the eleventh day, and a mouse unit is defined as the total amount of material required per mouse to ensure lobule-alveolar growth, comparable with that of glands taken from mice four to eight days pregnant, in $50 \pm 10\%$ of a group of ten test animals Under these revised conditions the test is far more sensitive and a clear relationship exists between the dose of pituitary material injected and the percentage of positive responses obtained A parallel is thus offered to the proportionality shown when graded doses of progesterone and some other related compounds are given, together with estrogen, in place of the pituitary injections (94)—circumstantial evidence that the lobule alveolar factor is secreted as a direct result of progesterone activity An attempt to explain this sensitizing action of estrogens within the framework of the mammogen theory has led to the suggestion (98) that it might be due to an accessory and direct action of the estrogen on the stromal tissue of the mammary gland, whereby an increased vascularity and hyperemia is caused, leading to a greater permeability of the blood vessels and a heightened mammogen concentration in the region of the developing parenchyma This view is supported by the demonstration of a similar function of estrogens in other fields—*e g* , the hyperemic reaction of the uterus (53), or the sexual skin of monkeys (11) It was also thought that estrogens might, in addition, have a special direct sensitizing effect on the mammary gland which would make it more responsive to stimulation by mammogenic factors There is evidently a close connection between the ability of estrogens to

enhance the effect of the lobule alveolar factor in intact mice and their action in promoting an increased duct response to various pituitary preparations in hypophysectomized mice (40), and the alternative possibility that in both cases it is the pituitary preparation which is sensitizing the mammary gland to the direct action of estrogens must not be overlooked

An attempt to characterize the lobule alveolar factor has shown that it is probably protein in nature but distinct from the lactogenic thyrotrophic, and gonadotrophic hormones (92), though this report must be considered in its relation to the evidence presented by Lyons (81) on the ability of purified lactogenic hormone preparations to maintain a normal duct system with a few alveoli in hypophysectomized female rats and of crude lactogenic preparations (containing also adrenotrophin and growth hormone) to cause an incomplete lobule alveolar development in similarly operated animals No other data are available to test its relation to the growth, adrenotrophic, or mammary duct growth hormones In view of the possibility of adrenocortical hormone participation in lobule alveolar growth, some measure of the adrenocorticotrophic hormone content of pituitary preparations assayed for mammogen II activity would clearly be of interest in order to establish that pituitary lobule-alveolar activity is not in fact an indirect function of the gland mediated by the cortical steroids This view receives some support from the work of Cowie and Folley (13) showing that improved duct and alveolar development follows the treatment of castrated male rats with ox anterior pituitary extracts provided only that the adrenal glands are not removed No difference in the responses of adrenalectomized and nonadrenalectomized female rats given similar treatment was observed however That the alveolar growth is not caused by the presence of progesterone in the anterior pituitary extracts has been shown by Trentin *et al* (152)

We may note in this connection that since duct growth has been observed in normal and castrate male mice following treatment with progesterone alone (37), adrenal participation might also be contributory to positive responses in the mammogen I assay

An interesting application of the mammogen II assay was reported by Mixner and Turner (97), which might seem to offer independent evidence of the validity of the mammogenic theory An increase of 10°C from 25° to 35° in the temperature to which the mice were subjected during assay was found to cause a great decrease in the response to injections of progesterone and estrogen, whereas the response to pituitary extract and estrogen was unaffected The authors interpret these results as indicative of a decreased ability of the mouse pituitary to secrete mammogen II in response to progesterone stimulation at the

higher temperature It is a pity, however, that the percentage positive responses at the lower temperature—progesterone treatment, 51 7, pituitary treatment, 86 7—were not more nearly comparable

The large body of facts now accumulated having relevance to the mammogen theory permits no final statement to be made of its validity One might hope for further enlightenment to be shed on this rather confused picture if experiments were conducted on assay animals subjected to adrenalectomy before pituitary preparations were tested Particularly difficult to explain by the tenets of the mammogen theory are the many instances reported of localized glandular development following injection of single mammae with ointments containing estrogens MacBryde (86) noted greater growth in human breasts so treated compared with the contralateral control breasts treated with the ointment base only Confirmatory reports were published by Lyons and Sako (85), *who, using young male rabbits, noted greater duct growth in the* estrogen-treated glands, in one case, although the same differential growth effect was found, the control gland too had developed to a certain extent, presumably indicating a better absorption of the estrogen in this animal or alternatively a greater sensitivity to the hormone (Fig 5) Speert (147) *and Chamberlin et al (11) have demonstrated identical* effects for young male monkeys, the latter authors drawing attention to their resemblance to the localized responses following the application of estrogens to the sexual skin of *Macaca mulatta* The use of gonadectomized male and female guinea pigs (110) has shown that in this species too, unilateral growth follows unilateral percutaneous administration of the hormone

In view of this general agreement regarding the action of locally applied estrogen in normal or castrated animals, the observations of Leonard and Reece (70) on the effect of similar treatment given to castrated, hypophysectomized rats are of great interest in their bearing on the validity of the mammogen theory In no case in which hypophysectomy was complete was any mammary growth seen in the estrogen-injected gland or its control, a fact clearly arguing some pituitary involvement Rats weighing, apparently, about 100 g were used in these experiments and treatment was delayed for three weeks following the operations, it would be interesting, in consequence of a later report (67), in which a greater response to estrogen injections in seventy-day-old rats was demonstrated when treatment was begun immediately after hypophysectomy rather than at the end of a postoperative recovery period if the work could be repeated observing these more favorable conditions

The suggestion that a local hyperemia in those glands injected with

estrogen conditions the differential response in normal animals has been put forward by Lewis *et al* (71) and by Mixner and Turner (98) to explain the unilateral effect They point out that mammogen I was

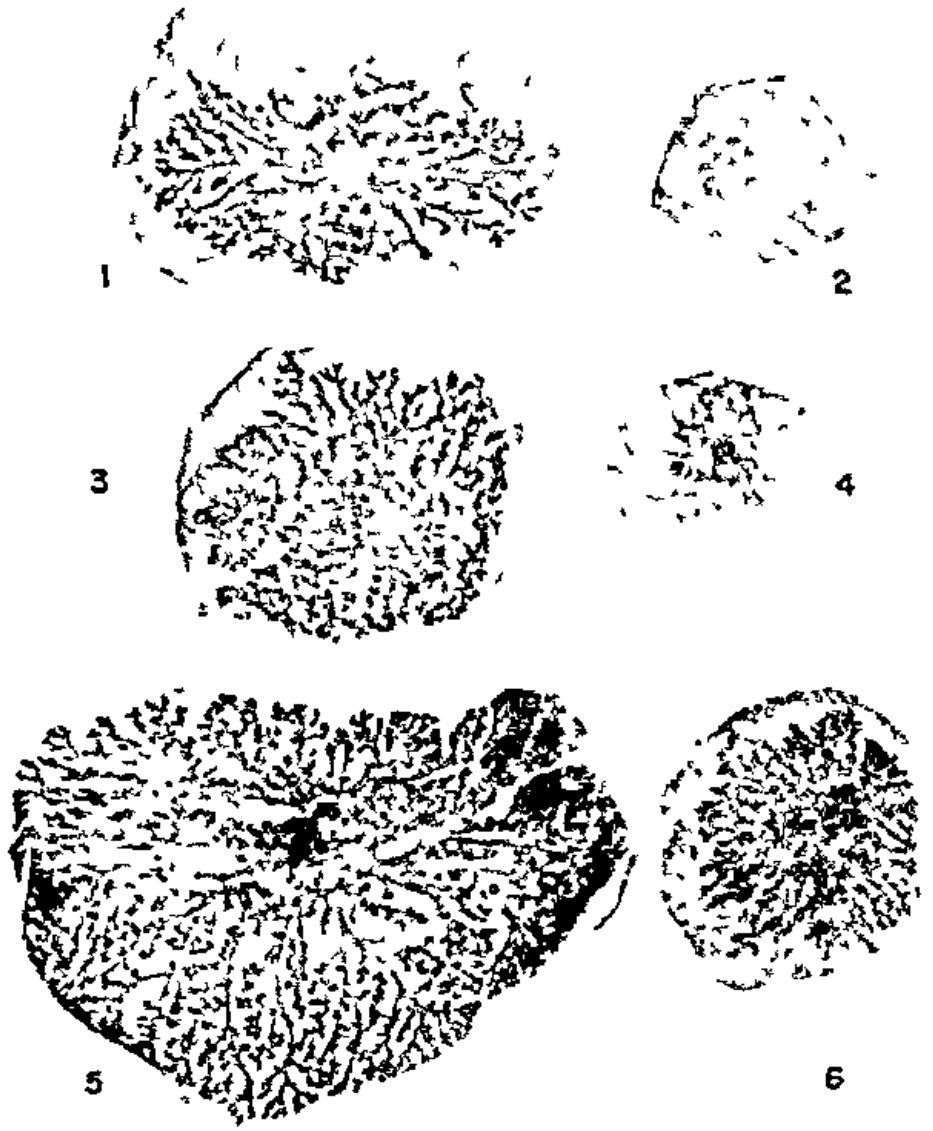


FIG 5—Mammary glands from left (1 3 5) and right (2,4 6) sides of three male rabbits Left glands injected for 25-day periods with estrone in sesame oil, right glands injected over the same periods with sesame oil alone (× 1 5) (From Lyons and Sako, 85)

found present, at least in cattle hypophyses, at all stages studied and that a local hyperemic condition might allow an enhanced effect of the mammogen, leading to more pronounced hyperplasia of the treated gland If this were so, rubefacients other than estrogen might also allow

increased mammogen activity when applied percutaneously; but attempts to demonstrate this using turpentine have so far been unconvincing (76,95)

It is clear that the results of unilateral stimulation, while difficult to reconcile with the theory of direct mammogen stimulation on the normal unsensitized gland (Fig 6A), harmonize better with the view that mammogens act directly on the estrogen sensitized gland (Fig 6B), and perhaps best of all with the view that growth follows direct estrogen

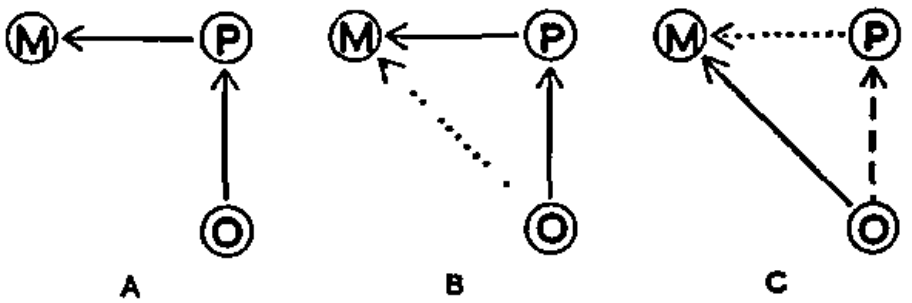


Fig 6 —Diagrammatic representation of possible interrelationships between ovarian and anterior pituitary hormones in the control of mammary growth M mammary gland P anterior pituitary O ovary For explanation see text

——————— trophic hormonal pathways
"sensitizing hormonal pathways
_ _ _ _ possible trophic hormonal pathway

action on a gland sensitized by some pituitary factor, for which "mammogen" would scarcely be an adequate name (Fig 6C) Whether or not the secretion of such a pituitary-sensitizing factor depends on estrogen stimulation of the pituitary would not affect the last interpretation of these facts

This synergistic, or "sensitizing" concept of ovarian hypophyseal interrelationship was first clearly enunciated by Reece and Leonard (131) as the most satisfactory interpretation of their own results, which showed that while growth hormone administered to hypophysectomized male rats would stimulate slight duct development, the simultaneous administration of estrogen greatly increased the effect The enhanced activity following estrogen treatment was accompanied by an adverse effect on the body weight of the rats, and in consequence the authors were unable to attach great significance to merely nutritional factors, and inclined rather to the view that " either estrogen facilitated the mammogenic effect of the growth hormone or that the growth hormone facilitated and was responsible for the effect of estrogen ' Remembering also Leonard s observation (67) on the ability of estrogens to stimulate mammary growth in hypophysectomized rats only when injections are begun immediately after hypophysectomy, it becomes still more likely

that pituitary involvement in mammary growth is a matter of inducing a sensitive condition in the undeveloped parenchyma rather than one whereby the secretion of specific mammogens directly, by themselves, causes an extension of tissue structures The gland may thus be "potentiated by pituitary factors rather than "developed by mammogens," such potentiation comprising an induced ability to respond to estrogenic substances Whether this ability is wholly dependent upon presensitization by the pituitary is not yet clear though the literature on the use of hypophysectomized animals in studies on mammary growth would suggest that it is so and that positive results following estrogen stimulation in such animals—where they cannot be explained by other factors already mentioned—may be due to an incomplete dissipation of the sensitizing effect at the time estrogen treatment was begun In particular the power of very small traces of residual pituitary tissue in incompletely operated animals to allow normal growth in response to estrogen treatment (46), is more plausibly explained by ascribing to the fragments the function of maintaining an already existing potentiating effect upon the mammary gland rather than that of producing under estrogen stimulation sufficient "mammogen to provide a normal mammary response by direct hormonal action Lewis and Turner (73) have reported mammogenic activity in cattle pituitaries at all stages of the animals' growth, even when no glandular development is taking place Possibly this anomaly could be explained by more rapid destruction of the circulating mammogen at certain periods when mammary growth is not taking place by a mechanism preventing mammogen release from the pituitary at these times or by the lack of sufficient estrogen to sensitize the gland to mammogen activity, but it may be doubted whether these explanations are as satisfactory as those based on the theory of sensitization by the pituitary For in the latter theory pituitary mediation is of itself latent only becoming apparent in the presence of estrogens, while the mammogen theory presupposes a hormone, or hormones, which if present could induce all the growth changes in the absence of other aid

On the other hand it is difficult to explain the absence of response in rats weighing more than 70 g and given estrogen immediately after hypophysectomy (67), by reference to the "sensitization" theory, though it may well be merely that the rate at which the potentiating effect disappears is linked with the animals' general development It is known for instance that for mice (32) successful estrogen treatment may be delayed for a longer period following hypophysectomy than is the case for rats It is also of interest to note that definite phases may be distinguished in the young intact rat regarding its mammary response to

estrogen, and that for the first two weeks of life estrogen has no demonstrable effect (4)

Smithcors and Leonard (145) have shown that for progesterone, too, although mammary stimulation followed treatment of the hypophysectomized rat, it was not optimal and only occurred if treatment began immediately after the hypophysectomy Combined estrogen and progesterone injections induced greater growth than either hormone alone in animals given immediate treatment, but growth never reached that shown by normal animals Gardner (32) also has reported mammary growth in hypophysectomized mice following treatment with progesterone, and observed improved stimulation when progesterone and estradiol dipropionate were given simultaneously Identical results were obtained when progesterone was replaced by desoxycorticosterone acetate in the latter experiments, in both cases the hormone, or combination of hormones, being administered for periods of 12–15 days and from 1–89 days after the hypophysectomy In the absence of estrogen, however, the mammary response was uncertain and only found in a small proportion of the subjects (progesterone 1 in 4, desoxycorticosterone acetate 2 in 7), and, in view of the entirely negative results of Chamorro (12) for adult hypophysectomized male mice given injections of desoxycorticosterone acetate, it might be of interest to know the age, sex, and time of injection relative to the operation, of the animals which gave these positive responses It seems possible that some factor depending on the sex of the animal might be influencing the results—perhaps the presence or absence of endogenous estrogens Apparently, however, mice, compared with rats, as already noted, suffer a slower postoperative change in the sensitivity of the mammary gland to various steroid hormones, for, in those experiments in which supplementary estrogen was also given, almost all the animals responded with mammary growth irrespective of the length of the postoperative period

Desoxycorticosterone has uniformly failed to induce new growth in the glands of hypophysectomized rats even when given with estrogen (70), or given alone under conditions favorable for estrogen or progesterone stimulation (145)

V. The Androgens and Mammary Growth

Evidence, summarized by Folley (21) and others, has frequently been obtained since the original experiments of Selye *et al* (141), that testosterone and also androgens semisynthetically produced may be active in causing mammary growth Alternative theories may thus be presented to explain the considerable mammary growth normally seen in males of certain species, such as the rat, and perhaps the abnormal growth—

gynecomastia—occasionally seen in males of other species, for example the human and the monkey Either these animals can produce substances having estrogenic activity—we may note that estrogens, possibly of adrenal origin occur in male urine (14)—in which case we could explain mammary growth in males in the same terms as for females, or growth is the result of androgenic stimulation and must be regarded as a distinct, though related, problem to that of normal female development Bottomley and Folley (7) have suggested that, in view of the experimental demonstration of the gynecogenic properties of the male sex hormones so far as mammary growth is concerned, there is no need to postulate the first alternative These authors themselves reported active duct proliferation in castrate male guinea pigs in response to Δ^5-*trans* androstenediol, testosterone propionate and 17-methyltestosterone, but in contrast to the results of Astwood *et al* (4) and Reece and Mixner (132) with rats injected with testosterone, alveolar development, and that very slight, was only seen after treatment with the first of these substances *cis* Androsterone and other androgens used had no clear proliferative effect on the mammary structures in agreement with earlier and later results obtained by other workers on the rat (113,127) van Heuverswyn *et al* (54) extended these results to mice finding extensive development of the duct system after normal animals were injected with testosterone, androstenedione, or dehydroisoandrosterone, but practically no effect with androsterone Dehydroandrosterone was later shown to be more active than testosterone in eliciting good alveolar responses from spayed female mice, when both hormones were given in conjunction with estrogen injections (99) Van Wagenen and Folley (166) found dilatation of the ducts of preadolescent ovariectomized female rhesus monkeys following injections of testosterone propionate, but no extension of the duct system or alveolar development unless alveoli were present before treatment was started (Fig 7)

The importance of estrogen in promoting an enhanced response to androgens was anticipated by the work of Laqueur and Fluhmann (66, see also 65), who found a dependence of the mammary response of adult rats to testosterone propionate upon the functional state of the ovaries at the time the first injection was given Animals whose treatment began during estrus gave a greater response, involving alveolar as well as duct development, the animals injected in diestrus showing mainly duct extension and only slight alveolar proliferation Only feeble responses were given, too, by immature rats, a result supported by the later work of Forbes (27) on the development of the mammary glands as the result of testosterone propionate pellet implantations

McEuen *et al* (89), using male rats, demonstrated that, as in the

case of estrogen stimulation, an intact hypophysis was necessary for any mammary response to injected androgens Endogenous androgen produced by injecting chorionic gonadotrophin was also ineffective in promoting growth after hypophysectomy, while the fact that injections of anterior pituitary gonadotrophic extracts permitted normal growth in the hypophysectomized animals to continue added to the evidence in favor of an essential pituitary factor participating in the androgenic growth effects These results, illustrating the importance of the pituitary, were confirmed by Noble (119) for the ovariectomized-hypophysectomized adult female and the hypophysectomized immature female rat treated with testosterone propionate

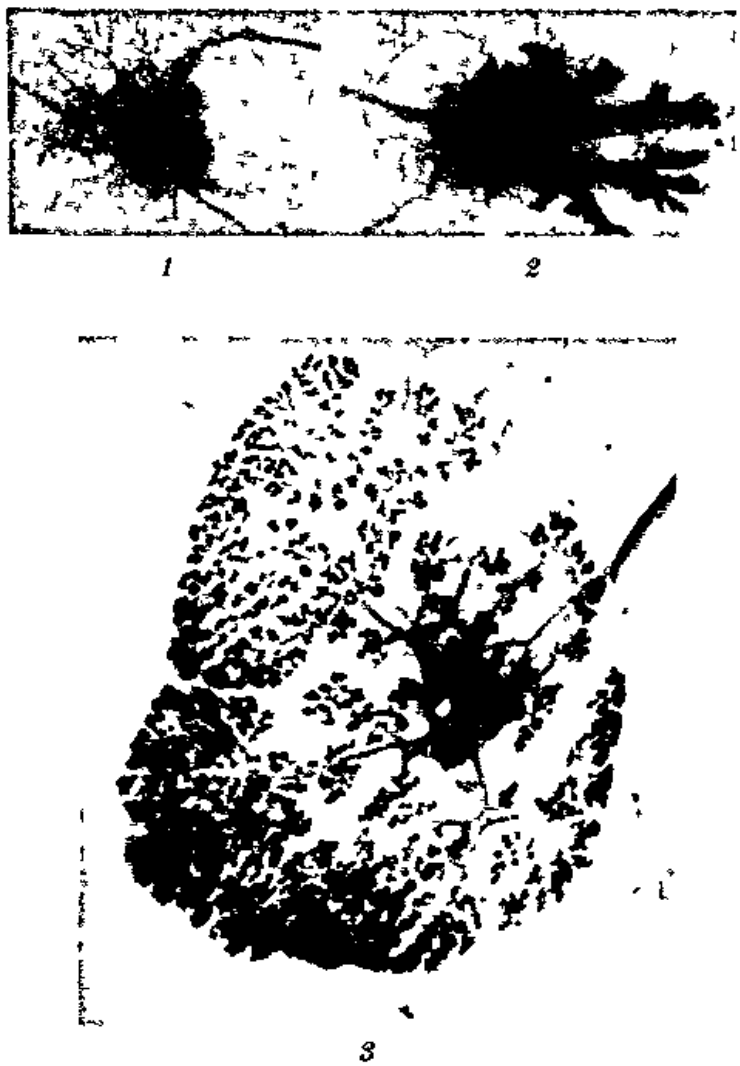
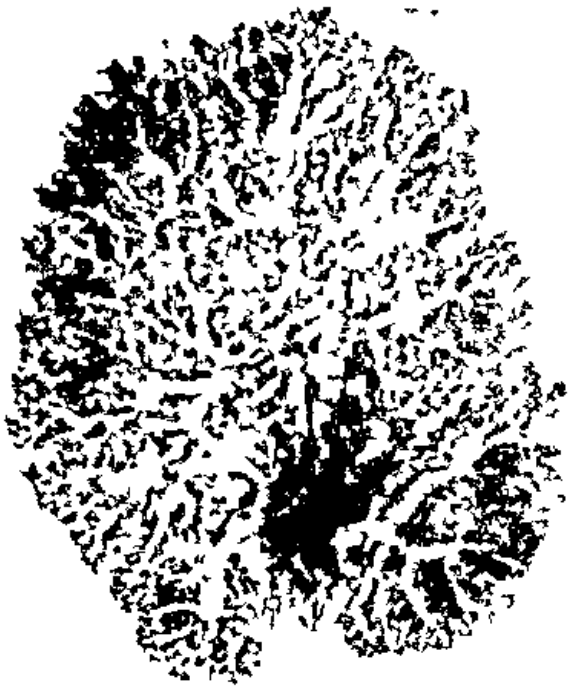


FIG 7 —Effect of testosterone propionate on mammary glands of preadolescent female rhesus monkeys *1* Control gland (monkey A) × 2. *2*. Gland after injection of 2000 mg testosterone propionate over 65 days (monkey A) × 2 *3* Control gland (monkey B) × 2



4





FIG 7—4 Gland after injection of 388 mg testosterone propionate over 65 days (monkey B) × 2 5 Photomicrograph of section of control gland (monkey B) × 6 5 6 Photomicrograph of section of gland after testosterone propionate injection (monkey B). × 6 8 (From Van Wagenen and Folley, 166)

VI. The Thyroid and Mammary Growth

Early experiments in which normal lactation, and presumably therefore normal mammary growth, was shown by thyroidectomized parturient bitches (17) and rats (115), or in which thyroidectomized and thyroidectomized, gonadectomized male and female rats were observed to give

marked mammary development following estrone injections (112) led to a tentative belief in the absence of any strongly characteristic effect of the thyroid on the growth of the mammary gland

This view was not in strict accord, however, with the results given by studies on the development of the gland under conditions of hyperthyroidism Weichert and Boyd (170), for instance, found a striking stimulation in the glands of thyroid-fed pregnant rats compared with normal pregnant controls, the degree of lobule alveolar formation being far superior by the ninth day of pregnancy, and the differential rate of development being maintained throughout the gestation period The authors expressed the opinion that the effect was due to endocrine factors rather than to any general influence of an increased metabolic activity A later report (171) revealed a difference in the histological picture presented by the mammary glands of rats in which pseudopregnancy had been induced by hyperthyroidism (169), and glands taken from animals in which pseudopregnancy was the result of sterile copulation with a vasectomized buck There was therefore an apparent ability on the part of the thyroid hormone to influence, whether directly or indirectly, the normal development of the gland The possibility of mediation via an effect on the ovary was perhaps supported by the inability of thyroid feeding to alter in any significant manner the glands of adult ovariectomized rats, but since the adult animals used failed to evince signs of mammary growth even when the thyroid feeding was supplemented by estrone injections, or in response to estrone injections alone, it is doubtful whether these experiments can be regarded as a critical test of ovarian participation in the particular thyroid function under discussion

The increase in the area of branching ducts in the glands of intact male mice when desiccated thyroid was added to the normal rations (34) did, however, again suggest gonadal involvement, since castrated male mice similarly treated failed to show such growth Since the adrenal glands also hypertrophied as the result of the thyroid treatment, it seemed that these glands too might be taking some part in the enhanced mammary reaction, but since a similar hypertrophy occurred in the castrated animals this explanation could not be regarded as wholly satisfactory

Mixner and Turner (100), under the conditions of their mammogen II assay test, found the lobule-alveolar response of spayed female mice given simultaneous progesterone and estrogen injections to be decreased by thyroidectomy and increased by thyroid feeding The authors were inclined to attribute these results to the stimulatory action of thyroxine on growth, whereby the normal optimal growth rate could be accelerated by increased thyroid activity, or decreased by hypoactivity of the gland

Confusing results obtained by Leonard and Reece (69) and Smithcors and Leonard (144) may conceivably have their interpretation in a distinction based on sex difference but the results in these two publications emphasize the ambiguities obscuring this branch of inquiry The first authors, using young female rats found that groups subjected to thyroidectomy, spaying and thyroidectomy or spaying thyroidectomy and estrogen injections all showed a greater degree of mammary growth—comprising thickened ducts and an increase in lateral and end buds—compared with appropriately treated unoperated control animals, simultaneous thyroxine injections given to rats treated otherwise as in the third, estrogen-injected group partially checked this differential growth Explanation of these results must at present remain purely conjectural, and, in view of the largely opposed results given by male rats treated similarly (144), some confirmatory evidence is needed The male animals showed inhibited duct development after thyroidectomy but an increased lobule-alveolar response on the limited but thickened duct systems present An interesting feature of the experiments was the augmented alveolar development when estrogen injections were given to thyroidectomized, castrated animals, equivalent to that given under similar conditions by testosterone propionate injections Possibly alterations in the metabolic pathways of steroids may be involved here, as the result of the thyroxine deprivation

Other studies, relating to the thyroidectomized bovine, have indicated that such operative treatment may result in subnormal mammary growth during pregnancy (148), and that udder development will not follow diethylstilbestrol treatment unless myxedematous symptoms are first removed by suitable supplementary thyroid feeding (125)

We are thus in the somewhat anomalous position of holding evidence that hypothyroidism and hyperthyroidism can both stimulate increased mammary growth, and further that hypothyroidism can cause duct-stunting effects *pari passu* with alveolar stimulation The various conjectural hypotheses put forward to explain the foregoing results are for the most part lacking all but the slenderest experimental support, but it would seem reasonable to suppose that two distinct mechanisms might well be contributing to the inadequately studied sequelae of abnormal thyroid functioning The first, necessarily linked in our consideration with inanition effects, depends upon alterations in the general systemic metabolism, and we may suppose that in this way growth changes, such as are implicated in mammary development, may be inhibited or accelerated despite conditions which, from the standpoint of hormonal activity, might be quite normal Secondly, the smooth interrelationship of the endocrine systems themselves may be upset, and we could formulate

a complicated series of influences at work which would purport the thyroid hormone to be active not merely directly or indirectly through one other gland, but at a multiple remove via intermediate effects on the gonads and the pituitary It would not then be surprising if paradoxical results should arise in investigating this problem, since the condition of these further participating glands might itself have a very important modifying effect on the outcome of any experiment

VII The Placenta and Mammary Growth

The various attempts to induce mammary growth experimentally, which have been enumerated above, all fail fundamentally to reproduce the conditions of most active natural growth—that is, growth during pregnancy—since all overlook certain concomitants of pregnancy which might be deemed to play some part in the growth process possible neural effects resulting from the distension of the uterus, for instance, hormonal effects deriving from the fetus or the uterine endometrium, or trophic secretions arising from the placenta We may suppose that some of these might exert at least a modifying effect upon the progress of mammary growth during normal pregnancy, and in fact there is a considerable amount of experimental evidence that the last of these influences—that of the placenta—has indeed a major importance

Selye *et al* (140) observed that, if the embryos and the ovaries were removed from rats in the middle of pregnancy, the mammary glands remained in a well-developed but nonsecretory condition, provided only that the placentae were retained intact This work was confirmed for the mouse by Newton and Lits (117), who further demonstrated a continuation of growth under placental influence during the second half of pregnancy in mice from which fetuses and ovaries had been removed This, it may be noted, is in conflict with the generally accepted view that proliferation of the parenchyma is completed during the first half of pregnancy in this species, and there would seem to be a distinct possibility that mammary hypertrophy—accompanying the incipient secretory activity of late pregnancy—or the inhibition of regressive changes might have been responsible for the "hyperplasia" which the authors inferred

The authors were able to conclude from their experiments that the presence of placentae in the uterus has a positive effect upon mammary development, which is independent of any ovarian action and which is not the result of uterine distension They were not able to define the trophic agency more accurately, but considered the direct action of an internal secretion of the placenta or endometrium to be a distinct possibility This view, as opposed to that of a neural or indirect hormonal

stimulus mediated by the pituitary, gained very strong support from the later studies of Gardner and Allen (35) and of Newton and Beck (116), whose results, to be considered together with the supplementary study of Newton and Richardson (118), showed that removal of the fetuses coupled with hypophysectomy at midpregnancy was only followed by involution of the mammary glands of mice if the placentae were also lost Those animals retaining adequate placental tissue were found to have a mammary development at the nineteenth day slightly in excess of that seen in normal mice on the twelfth day of pregnancy

Discussing the nature of this placental action the authors tentatively reject the theory of its dependence on a secretion of the ovarian hormones, an opinion which receives confirmation from the work of Lyons (82) on rats, spayed and hypophysectomized after one third of the gestation period had been completed, and then injected daily with estrone or progesterone or a combination of these hormones Only those animals receiving the last treatment, and a few injected with progesterone alone, showed the presence of placental tissue at full term, and these animals alone showed any extensive mammary development The other animals, in which resorption was complete, mainly showed a mammary system consisting of ducts only If, as has been shown under experimental conditions, the anterior pituitary is essential for the mammogenic activities of the ovarian hormones, it would be correct to conclude from Lyons' work that the placenta is able to assume those functions hitherto postulated for the pituitary, at any rate during the latter part of pregnancy, and further that, among the mechanisms responsible for mammary growth in the normal pregnant animal, the placenta may provide the "potentiating" mammogenic factor of which need is abundantly manifest Such a view would indicate that the pituitary plays a subsidiary role in this respect, having assumed an exaggerated importance in the consideration of hormonal mechanisms responsible for mammary growth merely by reason of the very abnormal conditions under which this problem has until recently been approached It seems quite possible that in most of the experiments showing the importance of pituitary mammogenic function this gland has been acting vicariously for the absent placentae of normal pregnancy We are reminded in this connection of a similar shared ability of the pituitary and chorionic tissues to secrete gonadotrophic substances

Still more recently Leonard (68) has presented further results testifying to the importance of placental tissue for mammary development or at any rate the prevention of mammary regression in the rat—at least during the later stages of pregnancy Rats were subjected to various operative procedures at the thirteenth day of pregnancy, involving the

removal singly or in all possible combinations of the pituitary, the ovaries, placentae, or fetuses It was observed that whatever the surgical removals might otherwise be, the retention of the placentae was never associated with any marked regression of the mammary gland, but usually with active development, while removal of the placentae, even when ovaries and pituitary were kept intact, invariably led to severe regression of the gland There was, however, fairly clear evidence that the placental mammogenic effect was enhanced in the presence of the pituitary and ovaries and somewhat reduced by their removal The author concluded that his results "indicate that the placenta of the rat is an endocrine organ and that the active principle(s) work synergistically with hormones of the hypophysis and ovaries to control mammary growth during the second half of pregnancy"

Another explanation of these results, in which the pituitary and the placenta are envisaged as serving separate ends, is the assumption that continued mammary growth in the presence of placental tissue and following hypophysectomy is due to a continuance for a period after operation of the pituitary-sensitizing effect upon the mammary gland, and that the placental role is not one of growth stimulation at all, but rather one of maintaining in functional state the nonsecreting (insofar as copious postparturient lactation is absent) but potentially secretory tissue Such a view would be consonant with the suggestion, already tentatively advanced, that the placenta might prove an agent of mammary hypertrophy but not of true hyperplasia

VIII Comparative Aspects of Mammary Growth Control

Although in the earlier part of this chapter the rat was taken as a prototype to illustrate the changes in mammary growth throughout the stages of a mammalian life cycle with several fairly characteristic sexual manifestations, it will have become clear that this generalization, made for convenience, will necessarily have to be modified in greater or less degree as one passes in review from species to species, by reason of the variations encountered in the underlying sex histories of different animals This comparative aspect of the problem has been well reviewed by Turner (156), and it is here intended merely to consider the fundamental secretory relationships which may be held responsible for the various types of development observed

Despite the recent evidence indicating a complex, multiple glandular control of mammary growth, it remains a basic postulate of all theories that growth results from hormonal stimuli initially set in train by one or both of the ovarian hormones, and, although the simple theory of direct stimulation of the gland by estrogens or progesterone may have to be

supplemented by other views, ovarian quiescence or activity does in fact, whether directly or indirectly, control the proliferation of ducts and lobules This control, we have already seen, may be shared, in a degree to which we can as yet give no quantitative estimate, by the adrenal gland, but in all probability only by virtue of the occurrence in that organ of substances identical with, or very closely akin to, the ovarian hormones themselves From this dependence upon ovarian function spring the possibilities for wide variations in both the quantitative and qualitative aspects of normal mammary development in different species, and particularly is this so in those stages of growth, between puberty and the first pregnancy, when fortunately mammogenic effects may be observed most unambiguously in relation to estrous or menstrual cycles

By contrast with the rat or the mouse in which the luteal phase is usually considered very short, or even absent, it might be expected that animals having estrous cycles with a definite luteal phase such as the cow, for which the normal cycle is of 21 days, or primates, with a menstrual cycle of about 28 days, would show a relatively greater alveolar development during the course of each cycle in response to the presence of an actively secreting corpus luteum for considerable periods Such development has been observed, and, since alveolar development was never found in rhesus monkeys which had undergone anovulatory cycles but only in those showing clear evidence that ovulation had occurred the importance of a lengthy luteal phase for this type of development during the sexual cycle was substantiated for this species (22) However, we may suppose that the presence of ripening follicles at this time will be continually affecting the progesterone estrogen ratio, rendering it optimal for mammary growth only for a small proportion of the time during which an active corpus luteum is present The conditions are thus not so favorable for growth as those observed during pseudopregnancy in some other mammals when a prolonged existence of the corpus luteum, in the absence of further follicular growth, does lead to the formation of relatively more extensive lobule-alveolar tissue In the rabbit, for instance, very extensive growth of the mammary alveolar system has been observed after sterile mating (1,49), while the complete mammary development following spontaneous infertile ovulation in the dog, is also linked closely with the persistence of the corpus luteum in this species during metestrus (91)

Another peculiarity which may be noticed in the rabbit is an absence of the waxing and waning effect in duct growth frequently seen in animals experiencing a regular succession of estrous cycles The rabbit shows a more steady growth in the period preceding its first pregnancy, correlated no doubt with the state of continuous estrus and a less fluctuating estrogen secretion

Apart from differences thus based on ovarian rhythms there remain the possibilities of variations in the actual hormonal stimuli required to give rise to mammary tissue, and though these agencies would appear to be the same in a qualitative sense for most species studied—and in all probability for all—it may well transpire that wide quantitative divergencies exist in the relative amounts of the hormones needed for optimal growth and in the relative importance of the contributions of individual glands toward this end

IX The Control of Nipple Growth

In contrast to the complexities of the hormonal control of the parenchymatous tissue of the mammary gland, it has become increasingly apparent that nipple, or teat growth is governed by simpler physiological mechanisms, and, although a variety of sex hormones can effect this development, their action in all cases would seem to be direct and not supplemented or modified by any pituitary function Thus Lyons and Pencharz (84) have found that the nipples of male guinea pigs show very much the same rate of growth following estrogen injection, whether or not the animals are previously subjected to hypophysectomy, and despite the fact that development of the mammary gland could only be obtained in the intact animals These results were confirmed and extended to include the female guinea pig by Gomez and Turner (43) For the male of this species Bottomley and Folley (7) demonstrated the dependence of teat growth on the endocrine activity of the testes, since castration stopped growth, in the young intact animal growth was found to proceed isometrically, keeping pace with the rate of body growth in general These authors tested a number of androgens for their power to cause accelerated growth of the teat in both normal and castrated guinea pigs and noted the greater efficacy of the unsaturated androgens in this respect Further evidence of the absence of any pituitary function in nipple growth was given by Noble (119), who found that this growth in the female rat, both adult and immature, was promoted by testosterone propionate injections irrespective of any previous hypophysectomy or ovariectomy This gynecogenic action was accordingly attributed by the author to direct stimulation of the nipple by the androgen

There are distinct indications that, with teat growth as for mammary gland development, species differences may deny us any thoroughly comprehensive explanation of the hormonal mechanisms involved, for Folley *et al* (26) have failed to observe any cessation of normal teat growth following castration in the young male goat This may suggest an altered sensitivity to androgens *vis-á-vis* the male guinea pig, or alter-

natively the presence of subsidiary mechanisms—involving possibly the adrenal gland—which can be called into action when the normal stimulating hormone source is removed The authors' demonstration of phases in teat growth in the caprine, coinciding with the onset and cessation of the breeding season, is of great interest since it implies an inhibitory function of progesterone, or of estrogen in large amounts, upon the rate of growth observed during anestrus Unlike the young male which shows an isometric teat growth rate, the female goat exhibits positive allometry at an early age The facts presented above for this species, together with the observation that positive allometric growth can be induced in males by appropriate treatment with estrogen, suggest that a dual mechanism may be responsible for normal growth in the female—one part, whose nature remains unknown, being responsible for a basal isometric rate of development as in the normal male and the other, probably a direct estrogen action, causing a superposed accelerating effect and responsible for the observed allometry Cessation of teat growth in the breeding season could then be explained by inhibition of the first "isometric" mechanism involving as a dependent effect failure of the second estrogenic stimulation

In conclusion reference may be made to numerous papers by Jadassohn and co-workers on the so-called "nipple-test"—differential growth effects produced by various sex hormones, when applied to the nipples of guinea pigs (58,59)

REFERENCES

1 Ancel, P , and Bouin, P *J physiol path gén* **13**, 31 (1911)
2 Anselmino, K J , Herold, L , and Hoffmann, F *Zentr Gynäkol* **59**, 963 (1935)
3 Asdell, S A , and Seidenstein, H R. *Proc Soc Exptl Biol Med* **32**, 931 (1935)
4 Astwood, E B , Geschickter, C F , and Rausch, E O *Am J Anat* **61**, 373 (1937)
5 Beall, D , and Reichstein, T *Nature* **142**, 479 (1938)
6 Bonser, G M *J Path Bact* **42**, 169 (1936)
7 Bottomley, A C , and Folley, S J *Proc Roy Soc London* **B126**, 224 (1938)
8 Burrows, H *J Path. Bact* **42**, 161 (1936)
9 Butcher, E O *Proc Soc Exptl Biol Med* **42**, 571 (1939)
10 Callow, R. K , and Parkes, A S *J Physiol* **87**, 28P (1936)
11 Chamberlin, T L., Gardner, W U , and Allen, E *Endocrinology* **28**, 753 (1941)
12 Chamorro, A *Compt rend soc biol* **133**, 546 (1940)
13 Cowie, A T , and Folley, S J *Nature* **154**, 302 (1944)
14 Dingemanse, E , Laqueur, E., and Mühlbock, O *ibid* **141**, 927 (1938)
15 Dodds, E C , Golberg L , Lawson, W , and Robinson, R *Proc Roy Soc London* **B127**, 140 (1939)
16 Doisy, E A , Thayer, S A , and van Bruggen, J T *Federation Proc* **1**, 202 (1942)
17 Dragstedt, L R , Sudan, A C , and Phillips, K *Am J Physiol* **69**, 477 (1924)

18 Emmens, C W. *Med Research Council (Brit) Special Rept Ser. No 234* (1939)
19 Emmens, C W. *J Endocrinol* **2**, 444 (1940–1941).
20 Engelhart, E *Klin Wochschr* **9**, 2114 (1930)
21 Folley, S J *Biol Rev Cambridge Phil Soc* **15**, 421 (1940)
22 Folley, S J , Guthkelch, A N , and Zuckerman, S *Proc Roy Soc London* **B126**, 469 (1939)
23 Folley, S J , and Malpress, F H *J Endocrinol* **4**, 1 (1944).
24 Folley, S J , and Malpress, F H *ibid* **4**, 23 (1944)
25 Folley, S J , Scott Watson, H M , and Bottomley, A C *J Physiol* **98**, 15P (1940)
26 Folley, S J , Scott Watson, H M , and Bottomley, A C *J Dairy Research* **12**, 241 (1941)
27 Forbes, T R *Endocrinology* **30**, 765 (1942)
28 Fredrikson, H *Acta Obstet Gynecol Scand* **19** (Suppl 1), 1 (1939)
29 de Fremery, P *J Physiol* **87**, 50P (1936)
30 de Fremery, P *Arch néerland zool* **3** (Suppl), 48 (1938)
31 Freyer, M E , and Evans, H M *Anat Record* **25**, 108P (1923)
32 Gardner, W U *Proc Soc Exptl Biol Med* **45**, 835 (1940)
33 Gardner, W U *Endocrinology* **28**, 53 (1941)
34 Gardner, W U *ibid* **31**, 124 (1942)
35 Gardner, W U , and Allen, E *Anat Record* **83**, 75 (1942)
36 Gardner, W U , Diddle, A W , Allen, E , and Strong, L C *ibid* **60**, 457 (1934)
37 Gardner, W U , and Hill, R T *Proc Soc Exptl Biol Med* **34**, 718 (1936)
38 Gardner, W U , Smith, G M , and Strong, L C *ibid* **33**, 148 (1935)
39 Gardner, W U , and Van Wagenen, G *Endocrinology* **22**, 164 (1938)
40 Gardner, W U , and White, A *Proc Soc Exptl Biol Med* **48**, 590 (1941)
41 Gardner, W U , and White, A *Anat Record* **82**, 414 (1942)
42 Gomez, E T *Endocrinology* **31**, 613 (1942)
43 Gomez, E T , and Turner, C W *Proc Soc Exptl Biol. Med* **34**, 320 (1936)
44 Gomez, E T , and Turner, C W. *Missouri Agr Expt Sta Research Bull* No **259** (1937)
45 Gomez, E T , and Turner, C W *Proc Soc Exptl Biol Med* **37**, 607 (1938)
46 Gomez, E T , Turner, C W , Gardner, W. U , and Hill, R. T *ibid* **36**, 287 (1937)
47 Gomez, E T , Turner, C W , and Reece, R P *ibid* **36**, 286 (1937)
48 Greep, R O , and Stavely, H E *Endocrinology* **29**, 18 (1941)
49 Hammond, J , and Marshall, F H A *Proc Roy Soc London* **B87**, 422 (1914)
50 Hammond, J , Jr , and Day, F T *J Endocrinol* **4**, 53 (1944)
51 Hartman, C G , and Speert, H *Endocrinology* **29**, 639 (1941)
52 Haterius, H O *Proc Soc Exptl Biol Med* **25**, 471 (1928)
53 Hechter, O , Krohn, L , and Harris, J *Endocrinology* **29**, 386 (1941)
54 van Heuverswyn, J , Folley, S J , and Gardner, W U *Proc Soc Exptl Biol Med* **41**, 389 (1939)
55 Hoffman, M M , Kazmin, V E , and Browne, J S L *J. Biol Chem* **147**, 259 (1943)
56 Horwitt, B N , Dorfman, R I , Shipley, R A , and Fish, W R *J Biol Chem* **155**, 213 (1944)
57 Iscovesco, H *Compt rend soc biol* **73**, 104 (1912)
58 Jadassohn, W , and Fierz-David, H E *Vierteljahrsschr naturforsch Ges Zurich* **88**, 1 (1943)

59 Jadassohn, W , Uehlinger, E , and Margot, A *J Investigative Dermatol* **1**, 31 (1938)
60 Jeffers, K R. *Am J Anat* **56**, 257 (1935)
61 Jeffers, K R. *ibid* **56**, 279 (1935)
62 Lane Claypon, J E , and Starling E H *Proc Roy Soc London* B77, 505 (1906)
63 Laqueur, E , Borchardt, E , Dingemanse, E , and De Jongh, S E *Deut med Wochschr* **54**, 465 (1928)
64 Laqueur, E , de Jongh, S E , and Tausk, M *ibid* **53**, 867 (1927)
65 Laqueur, G L. *Endocrinology* **32**, 81 (1942)
66 Laqueur, G L., and Fluhmann, C F *Endocrinology* **30**, 93 (1942)
67 Leonard, S L. *ibid* **32**, 229 (1943)
68 Leonard, S L. *Anat Record* **91**, 65 (1945)
69 Leonard, S L., and Reece, R P *Endocrinology* **28**, 65 (1941)
70 Leonard, S L., and Reece, R. P *ibid* **30**, 32 (1942)
71 Lewis, A A , Gomez, E T , and Turner, C W *ibid* **30**, 37 (1942)
72 Lewis, A A , and Turner, C W *Proc Soc Exptl Biol Med* **39**, 435 (1938)
73 Lewis A A , and Turner, C W *Missouri Agr Expt Sta Research Bull* No 310 (1939)
74 Lewis, A A , and Turner, C W *J Dairy Sci* **24**, 845 (1941)
75 Lewis, A A , and Turner, C W *Endocrinology* **30**, 585 (1942)
76 Lewis, A A , and Turner, C W *ibid* **30**, 985 (1942)
77 Lewis, A A , and Turner, C W *ibid* **31**, 520 (1942)
78 Lewis, A A , and Turner, C W *J Dairy Sci* **25**, 895 (1942)
79 Long, J A , and Evans, H M *Mem Univ Calif* **6**, 1 (1922)
80 Lyons, W R. *Proc Soc Exptl Biol Med* **51**, 308 (1942)
81 Lyons, W R. Essays in Biology Univ California Press Berkeley and Los Angeles, 1943, p 315
82 Lyons, W R *Anat Record* **88**, 446 (1944)
83 Lyons, W R., and McGinty, D A *Proc Soc Exptl Biol Med* **48**, 83 (1941)
84 Lyons, W R., and Pencharz, R. I *ibid* **33**, 589 (1935–1936)
85 Lyons, W R , and Sako, Y *ibid* **44**, 398 (1940)
86 MacBryde, C M *J Am Med Assoc* **112**, 1045 (1939)
87 MacDonald, I G *Surg Gynecol Obstet* **63**, 138 (1936)
88 McEuen, C S , Selye, H , and Collip, J B *Proc Soc Exptl Biol Med* **35**, 56 (1936–1937)
89 McEuen, C S , Selye, H , and Collip, J B *ibid* **36**, 213 (1937)
90 Maeder, L M A *Am J Anat* **31**, 1 (1922)
91 Marshall, F H A , and Halnan, E T *Proc Roy Soc London* B89, 546 (1917)
92 Mixner, J P , Bergman, A J , and Turner, C W *Endocrinology* **31**, 461 (1942)
93 Mixner, J P , Lewis, A A , and Turner, C W *ibid* **27**, 888 (1940)
94 Mixner, J P , and Turner, C W *ibid* **29**, 324 (1941)
95 Mixner, J P , and Turner, C W *Proc Soc Exptl Biol Med* **46**, 437 (1941)
96 Mixner, J P , and Turner, C W *ibid* **47**, 453 (1941)
97 Mixner, J P , and Turner, C W *ibid* **48**, 443 (1941)
98 Mixner, J P , and Turner, C W *Endocrinology* **30**, 591 (1942)
99 Mixner, J P , and Turner, C W *ibid* **30**, 706 (1942)
100 Mixner, J P , and Turner, C W *ibid* **31**, 345 (1942)
101 Mixner, J P , and Turner, C W *Missouri Agr Expt Sta Research Bull* No 378 (1943)
102 Myers, F J , and Myers, J A *Anat Record* **21**, 74 (1921)

103 Myers, J A *Am J Anat* **19**, 353 (1916)
104 Myers, J A *Anat Record* **13**, 205 (1917)
105 Myers, J A *Am J Anat* **25**, 395 (1919)
106 Nathanson, I T, Shaw, D T, and Franseen, C C *Proc Soc Exptl Biol Med* **42**, 652 (1939)
107 Nelson, W O *Physiol Revs* **16**, 488 (1936)
108 Nelson, W O *Am J Anat* **60**, 341 (1937)
109 Nelson, W O *Anat Record* **73**, 39 (Suppl) (1939)
110 Nelson W O *Am J Physiol* **133**, P397 (1941)
111 Nelson, W O, Gaunt, R, and Schweizer, M *Endocrinology* **33**, 325 (1943)
112 Nelson, W O, and Hickman, J *Proc Soc Exptl Biol Med* **36**, 828 (1937)
113 Nelson, W O, and Merckel, C G *ibid* **36**, 823 (1937)
114 Nelson, W O, and Pfiffner, J J *Anat Record* **51**, 51 (1931)
115 Nelson, W O, and Tobin, C E *Endocrinology* **21**, 670 (1937)
116 Newton, W H, and Beck, N *J Endocrinol* **1**, 65 (1939)
117 Newton, W H, and Lits, F J *Anat Record* **72**, 333 (1938)
118 Newton, W H, and Richardson, K C *J Endocrinol* **2**, 322 (1940)
119 Noble R L *ibid* **1**, 184 (1939)
120 Parkes A S *The Internal Secretions of the Ovary* Longmans, Green London, New York and Toronto, 1929
121 Parkes, A S *Proc Roy Soc London* **B104**, 189 (1929)
122 Parkes A S *ibid* **B107**, 188 (1930–1931)
123 Parkes, A S, and Glover, R E *J Endocrinol* **4**, 90 (1944)
124 Petersen W E *Physiol Revs* **24**, 340 (1944)
125 Petersen, W E, Knodt, C B, Ludwick, T M, and Pomeroy, B S *Proc Soc Exptl Biol Med* **57**, 332 (1944)
126 Pincus, G, and Pearlman, W H *Vitamins and Hormones* **1**, 293 (1943)
127 Reece R P *Proc Soc Exptl Biol Med* **46**, 265 (1941)
128 Reece, R P *ibid* **52**, 145 (1943)
129 Reece, R P, and Bivins, J A *ibid* **49**, 582 (1942)
130 Reece, R P, and Leonard, S L *ibid* **42**, 200 (1939)
131 Reece R P, and Leonard, S L *Endocrinology* **29**, 297 (1941)
132 Reece, R P, and Mixner, J P *Proc Soc Exptl Biol Med* **40**, 66 (1939)
133 Reeder C F, and Leonard S L *ibid* **55**, 61 (1944)
134 Richter, I *Anat Anz* **66**, 145 (1928)
135 Roberts F L Papers from the Mayo Foundation and the Medical School of the University of Minnesota, Philadelphia and London **1**, 228 (1921)
136 Samuels L T Reinecke, R M, and Petersen, W E *Proc Soc Exptl Biol Med* **46**, 379 (1941)
137 Scharf G, and Lyons, W R *ibid* **48**, 86 (1941)
138 Selye, H *ibid* **43**, 343 (1940)
139 Selye H Browne, J S L, and Collip, J B *ibid* **34**, 472 (1936)
140 Selye H Collip, J B, and Thomson, D L *Endocrinology* **19**, 151 (1935)
141 Selye H McEuen, C S, and Collip, J B *Proc Soc Exptl Biol Med* **34**, 201 (1936)
142 Selye H, and McKeown, T *Anat Record* **60**, 323 (1934)
143 Selye H and McKeown, T *Proc Soc Exptl Biol Med* **31**, 683 (1934)
144 Smithcors J F, and Leonard, S L *Endocrinology* **31**, 454 (1942)
145 Smithcors, J F, and Leonard, S L *Proc Soc Exptl Biol Med* **54**, 109 (1943)
146 Speert, H *Bull Johns Hopkins Hosp* **67**, 189 (1940)

147 Speert, H *Science* **92**, 461 (1940)
148 Spielman, A A, Petersen, W E, and Fitch, J B *J Dairy Sci* **27**, 441 (1944)
149 Sutter, M *Anat Record* **21**, 59 (1921)
150 Szego, C M, and Samuels, L T *J Biol Chem* **151**, 599 (1943)
151 Trentin, J J, Lewis, A A, Bergman, A J, and Turner, C W *Endocrinology* **33**, 67 (1943)
152 Trentin, J J, Mixner, J P, Lewis, A A, and Turner, C W *Proc Soc Exptl Biol Med* **46**, 440 (1941)
153 Trentin, J J, and Turner, C W *Endocrinology* **29**, 984 (1941)
154 Turner, C W Sex and Internal Secretions Williams and Wilkins, Baltimore, 1932, p 544
155 Turner, C W The Comparative Anatomy of the Mammary Glands University Cooperative Store, Columbia, Mo, 1939
156 Turner, C W Sex and Internal Secretions 2nd ed Baillière, Tindall and Cox London, 1939, p 740
157 Turner, C W, and Allen, E *Anat Record* **55**, 80 (Suppl) (1932–1933)
158 Turner, C W, and De Moss, W R *Missouri Agr Expt Sta Research Bull* No 207 (1934)
159 Turner, C W, and Frank, A H *ibid* No 145 (1930)
160 Turner, C W, and Frank, A H *ibid* No 174 (1932)
161 Turner, C W, Frank, A H, Gardner, W U, Schultze A B and Gomez E T *Anat Record* **53**, 227 (1932)
162 Turner, C W, and Gomez, E T *Missouri Agr Expt Sta Research Bull* No 182 (1932)
163 Turner, C W, and Gomez, E T *ibid* No 206 (1934)
164 Turner, C W, and Gomez, E T *ibid* No 207 (1934)
165 Turner, C. W, and Schultze, A B *ibid.* No 157 (1931)
166 Van Wagenen, G, and Folley, S J *J Endocrinol* **1**, 367 (1939)
167 Walker, S M, and Stanley, A J *Proc Soc Exptl Biol Med* **48**, 50 (1941)
168 Weatherford, H L *Am J Anat* **44**, 199 (1929)
169 Weichert, C K, and Boyd, R W *Anat Record* **58**, 55 (1933)
170 Weichert, C K, and Boyd, R W *ibid* **59**, 157 (1934)
171 Weichert, C K, Boyd, R W, and Cohen, R S *ibid* **61**, 21 (1934–1935)

Chapter XVI

Hormonal Control of Lactation

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In a short review of the literature pertaining to mammary lobule alveolar growth in goats Mixner and Turner (187) make passing reference to the fact that " considerable milk secretion can occur from a well extended duct system ' This contention is supported by evidence which they themselves have derived from the goat, and which is appended to the review Most relevant is the record of one animal given daily injections of diethylstilbestrol, which produced a maximum milk yield of 56 ml per day and yet showed "a conspicuous lack of any degree of development of the lobule alveolar system " No doubt a very slight degree of alveolar development would be sufficient for such a low milk production but the result implies an important contribution by duct secretion to the total milk volume in this particular case Gardner *et al* (98) had previously observed a similar phenomenon in the rabbit, the duct systems of ovariectomized immature females and of males pretreated with estrogen both responding to pituitary lactogenic preparations with clear evidence of milk secretion Cytological data as reported in the previous chapter have also confirmed that lactation need not inevitably be associated with the presence of alveolar tissue However,

we may safely assume that, judged from its quantitative aspect normal milk secretion following pregnancy or pseudopregnancy is for all mammals overwhelmingly a property of the alveoli, and that duct secretion plays but an insignificant part in this process

The degree of lactation shown by experimental or normal parturient animals will consequently have particular reference to the lobule-alveolar development of the gland at the time of onset of lactation and, specific lactogenic factors apart, the process of milk secretion must be regarded as being basically a function of mammary development and, as such, largely dependent upon those hormonal mechanisms which we have already had under consideration Here, however, our attention is directed to those supplementary hormonal influences responsible for the initiation and maintenance of secretion from an adequately formed mammary parenchyma

I The Anterior Pituitary and Lactation

A General

The tentative ideas formerly held regarding the control of secretion from the developed mammary gland were largely suggested by the temporal relationship (confusing in many respects as we now know it to have been) between the start of copious lactation and the various changes in the genital system attendant upon parturition Lactation was seen as a passive process ensuing when the factors leading to the growth of the gland had completed their work, or alternatively it was thought that the growth mechanisms, which at that time were themselves only partly known, might, while operative, have an actual inhibitive effect on the processes of secretion

The demonstration by Stricker and Grueter (268) in 1928 of the ability of anterior pituitary preparations to initiate lactation in pseudopregnant rabbits, whether normal or ovariectomized on the tenth day of their pseudopregnancy, immediately brought about a change in scientific opinion on this problem It appeared that, in the presence of developed tissue, lactation could be evoked by a hitherto unsuspected pituitary factor By inference it followed that the onset of lactation in the normal animal could no longer be visualized as the result of the smooth, uninstrumented transition of a growth phase into a secretory phase in alveoli which had reached a certain favorable stage of development, or the removal of an inhibitory agent at parturition, but as due to the intervention at that time of a new stimulus having positive powers in its own right and without which the gland could not possibly function

The rabbit, for a variety of reasons, notably the ease with which

developed glands could be produced through pseudopregnancy and the dependence of alveolar growth upon the presence of functioning corpora lutea, has always been a popular animal for research on the biological properties of lactogenic extracts Stricker and Grueter in addition to the pioneer results already quoted reported in the same paper that immature rabbits failed to secrete milk following the injection of aqueous anterior pituitary extracts and thus first pointed to the need for a minimum morphological development before secretion could begin This last result was at variance with work published by Corner (39), who reported both mammary growth and copious lactation following the injection of an alkaline extract of whole sheep pituitary into spayed virgin rabbits whose ovaries when removed contained no trace of luteal tissue

These conflicting results provided the background to a controversial side issue concerning the power of pituitary lactogenic extracts to evoke mammary growth Gardner and Turner (99) for instance stimulated milk secretion in the involuted rabbit gland frequently equal in amount to that obtained after parturition but concluded that their extracts had no power to cause an increase in the number of alveoli originally present in the involuted glands, the effect being one of restoration to activity of alveoli that had perhaps stopped secreting but had not regressed too far for renewed activity under suitable stimuli In support of Corner's work, however, Lyons and Catchpole (161) and Asdell *et al* (9) reported mammary development following the injection of pituitary extracts having lactogenic properties into spayed virgin rabbits Photographs of whole mammary spreads in the former publication (Fig 1) are of considerable interest in this connection and it is clear that the matter, still one of considerable contemporary interest was at the time confused by the apparently conclusive evidence submitted by both parties to the dispute

The resolution of these differences may lie partly in the claim of Gardner *et al* (98) that duct secretion is responsible for the milky fluids produced from undeveloped glands treated with suitable pituitary preparations, and that hyperplasia of the gland is more apparent than real, the effect being rather one of duct hypertrophy and distension of the gland with the newly formed secretion It is doubtful however, whether this single explanation will suffice as a reinterpretation of the results of Lyons and Catchpole and it seems more likely that these authors did in fact get a true mammary hyperplasia after administering their pituitary extract but not only by virtue of any lactogenic principle or principles it may have contained, the presence of contaminating adrenocorticotrophic hormone in the extracts may be deemed highly probable (157,188)

and the formation of a more highly developed mammary parenchyma may have resulted from increased adrenal cortex activity (see Chapter XV) The separate identity of the lactogenic and adrenocorticotrophic principles has been established (157,188) and divergent results of different workers on the mammogenic properties of lactogenic preparations might be explained by the presence of the second principle in varying amounts in different extracts or, should their existence be established, the presence or absence of pituitary mammogens (see Chapter XV) in different preparations

In view of these alternative explanations the question of how far the pituitary lactogenic extracts themselves possessed mammary-growth-stimulating properties, by virtue of their lactogenic attributes, awaited the preparation of purer, and especially adrenocorticotrophin-free, extracts This, and much other work beside on the hormonal control of lactation, was made possible by the extensive investigations of Riddle and co-workers (236,237), who, making use of the earlier discovery of Riddle and Braucher (238) that the pigeon crop gland was activated to growth and secretion by suitable anterior pituitary extracts, demonstrated that the active principle of these extracts, to which they gave the name "prolactin," was also the factor causing milk secretion in the developed glands of mammals That its identity was separate from the other known pituitary hormones was also established and preparations having a relatively high degree of purity were obtained With the aid of such prolactin extracts Lyons (159), using his most elegant technique of intraduct injection, has recently claimed to have obtained definite evidence of localized hyperplastic changes in the alveoli of ovariectomized virgin rabbits pretreated with estrogen and progesterone The reported changes include a great increase in the number of epithelial cells forming the circumference of the alveoli, and the presence of mitotic figures in their epithelia Comparisons were made with control sectors from uninjected parts of the same glands (Fig 2) The author further states that "when full lactation has set in the secretory cells have to be constantly renewed either in their entirety or—as is more usually the case—in their supra-nuclear cytoplasm only" and implies that such reconstitution may well be a growth function of the lactogenic hormone The claim is made specifically by this author in a further publication (158) in which he suggests that the lactogenic principle " initiates in the estrin or estrin-progestin-prepared mammary gland a functional alveolar growth leading to lactation " Although not phylogenetically homologous with the changes seen in the developing parenchyma of the mammalian gland, the pigeon-crop stimulating reaction of the lactogenic hormone, to which we shall recur is witness to its ability to promote tissue growth as a

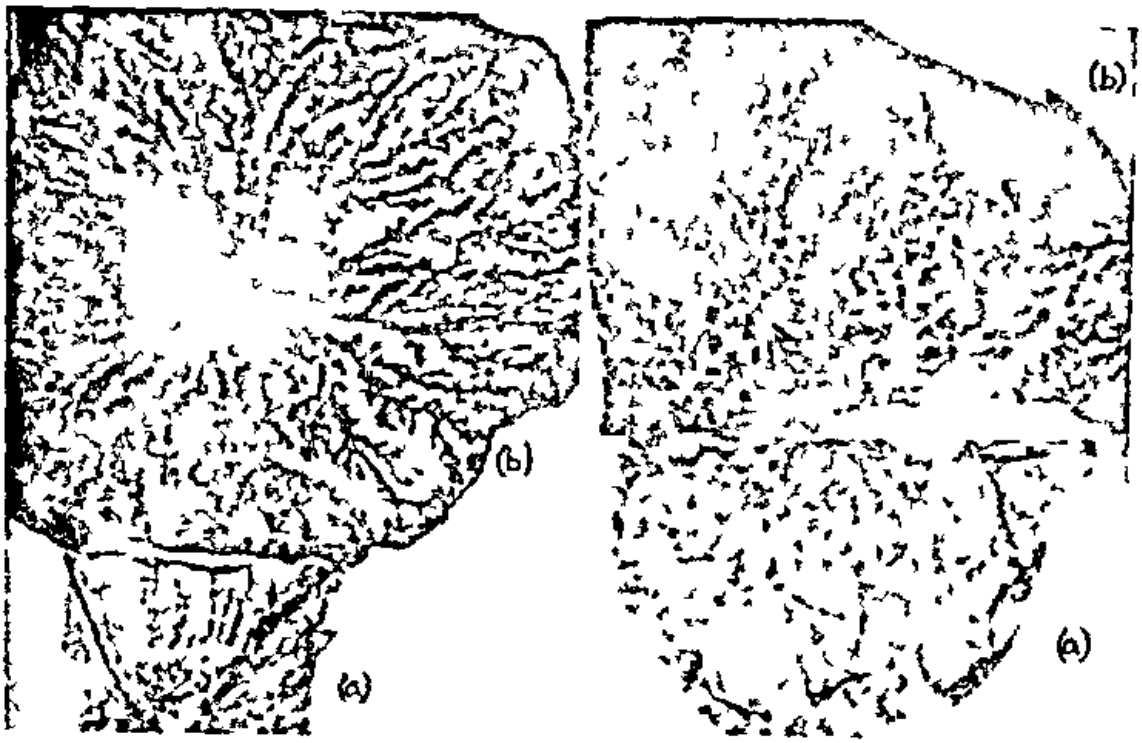


FIG 1 —(a) *Control rabbit mammary spreads compared with* (b) *spreads from the same animals removed after injections of lactogenic hormone preparations had been given* (From Lyons and Catchpole 161)

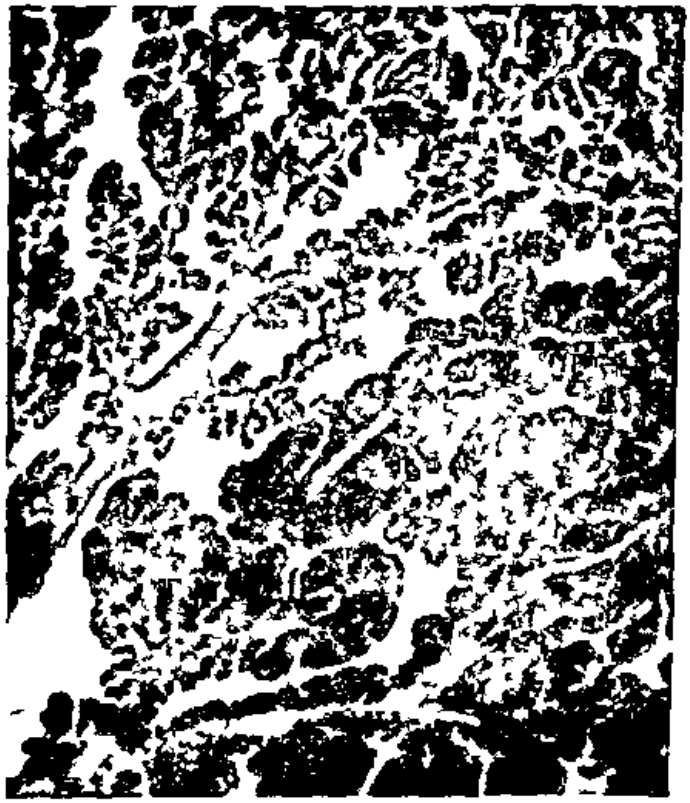


FIG 2 —Two adjacent sectors of a mammary gland of an ovariectomized rabbit pretreated with estrone and progesterone and then given prolactin into the main duct (lower left) of the bottom sector This sector alone lactated (×3 7) (*From Lyons 159*)

preliminary to the lactational response, and it might prove to be the case that the actual cytological changes postulated by Lyons have features in common with this parallel response

Lyons' conception is one of the utmost importance, implying as it does the existence of hormonal control of cellular rejuvenation or renewal in the lactating gland, but confirmation and a more precise definition of the significance of the action will be needed before this function of the lactogenic hormone can be accepted without reserve

One other hormonal mechanism may be mentioned here as possibly affecting the lactation response by superimposing a growth effect on the gland The luteotrophic hormone, which has the property of maintaining corpora lutea in an active secretory condition (11) has been identified by many workers with the lactogenic hormone (see page 798), if this is so, and should the animal injected with the hormone be intact and have luteal tissue present in the ovary, the stimulation would result in a release of progesterone which might promote glandular development conditioning an improved lactational response Reports dealing with this luteotrophic function of prolactin are more fully considered in a later section

Contrasting with the different views held on the growth-promoting potentiality of prolactin preparations there has been complete unanimity regarding their lactogenic powers, and for the rabbit, in addition to the reports already mentioned, Fredrikson (94) has observed milk secretion in spayed immature animals whose glands had been developed prior to the pituitary injections by treatment with estradiol monobenzoate and progesterone, while Anselmino *et al* (5) have reported lactation in male rabbits given similar conditioning treatment

These investigations were extended to other species with similar results so far as the lactogenic properties of fresh pituitary implants or extracts were concerned A study with the guinea pig as experimental subject was made by Nelson and collaborators (199,202), who initiated lactation in normal and castrate immature males and in spayed immature females all of which had been given preliminary treatment with an extract of sows' corpora lutea This extract developed the mammary glands, primarily owing to its estrogen content, and it was reported that such development with ovarian hormones was a necessary prerequisite for the lactogenic response to pituitary injections Such parenchymatous growth could equally well be given by ovarian transplants into males, either castrated or made experimentally cryptorchid These authors found no evidence of any power on the part of the pituitary extracts they used to cause growth of the guinea pig gland, and their report (200) of

lactation in adult virgin animals gonadectomized during estrus, following the injection of pituitary extract alone, may be taken as a probable indication of a certain degree of normal mammary growth in the animals before experimental treatment was begun Lyons and Catchpole (160), who obtained similar results with ovariectomized, mature virgin guinea pigs, also favored this explanation

The review of Nelson (193) and the bulletin of Gardner and Turner (100) may be consulted for references to the many parallel experiments carried out on other small mammals which substantiate the work on the rabbit and guinea pig The latter publication draws special attention to the greater difficulty experienced by early workers in getting lactational responses from the rat They themselves quote results illustrating this difficulty and conclude that differences observed between the rat and the rabbit may have their origin in more rapid and extensive involutionary changes occurring in the former species Thus for instance, mature parous rats treated after weaning with pituitary preparations may, in the absence of additional treatment to maintain the morphological elements of the gland, present a regressing parenchyma inadequate for a proper response to lactogenic preparations A supplementary effect may be a smaller quantitative significance of duct secretion in the rat compared with other species. That no fundamental qualitative difference exists is demonstrated by the work of Schultze and Turner (249), who were able to induce lactation in the mature multiparous rat and in immature rats given pituitary implants, provided the ovaries of the host animals remained intact and the pituitary implants, supplemented by the animals own endogenous secretions, were sufficiently rich in gonadotrophic activity

It is now clear that the need for progesterone stimulation in order to obtain alveolar development in the rat was frequently an unappreciated complication in the earlier experiments, which, together with the other factors already mentioned, presented a problem in comparative lactation that was in reality largely a problem of comparative mammary development Reece (220) has suggested that the "refractoriness" of the mammary glands of the pseudopregnant rat to prolactin may be due to the presence of some other essential hormone in insufficient amounts The role of other glands in lactation will be considered later, but Reece's own experiments in which prolactin or prolactin plus adrenocortical hormone preparations were given to such animals cannot be regarded as providing results strongly in support of this theory

The effect of prolactin in primates has been more difficult to assess experimentally, but despite conflicting clinical reports it may be confi-

dently assumed to be the same as in the small mammals already instanced. Allen *et al* (4), for example, induced lactation in monkeys treated with estrogen and lactogenic preparations or with the latter alone, finding however that successful treatment was correlated with sexual maturity or, in other words, dependent in all probability upon some measure of alveolar development in the glands With humans, experiment has been largely confined to attempts to increase a poor initial lactation following normal parturition, and in spite of the hazards of serious local reactions observed by some workers following injections (289) and some disappointing clinical reports (*e g* 266), there seems to be a hope of useful extended clinical application of the hormone in selected cases as soon as it becomes more readily available in the purified form Kenny and King (144) using ox and sheep pituitary preparations have reported 74% positive responses—milk increments of over 100 ml daily—in their treated group of 43 women, compared with 21% positive responses among the same number of controls treated by routine methods—breast pump, massage extra milk rations, and so forth These hopeful results have recently been confirmed by Wilson (290) and were themselves a confirmation of some earlier reports (*e g* 241) It would, however, be unwise to draw any premature conclusions from the very inadequate data already at hand, especially since the reports almost all deal with the problem of increasing a milk yield already established and, as will be shown later, there may be justifiable grounds for believing that this may depend on a phase of the hormonal pattern controlling lactation which presents subtle differences from that causing initiation of secretion from a nonsecreting gland, prolactin seems primarily concerned with the latter function It seems likely however, that in some cases of poorly established lactation a partial deficiency of prolactin will prove to be the correct diagnosis and injections with this hormone a successful therapy

Gradually, and for reasons which must be sufficiently obvious, studies on the biological effects of the lactogenic hormone have come to depend more and more on the use of dairy farm animals The goat and the cow are ideal subjects for responses primarily to be measured by changes in milk yields and they present the additional advantage that results obtained by their use have frequently a direct reference to actual practical problems of the dairy industry

Following the early application by Gruter and Stricker of anterior pituitary treatment to the cow (see below), Asdell reported the first results demonstrating the induction of lactation in a young female kid (7), and the arrest of the normal decline in the milk yields of parturient goats treated late in lactation (8) These cases, with repetitive experiments in which it was found that a positive effect on the milk yield was

an uncertain response more likely to be obtained from animals giving low rather than high yields at the time of injection, and further work illustrating the related fact that little effect either on maximum daily yield or persistence of lactation was to be expected when animals were injected at the peak of their lactation were all collected in a later memoir (9) The authors here supported the view that the effect at the peak of lactation was absent because the limiting factor at this point is normally one of mammary development, and that fruitful responses from anterior pituitary injections could only be expected when lactation was diminishing and then only in those animals for which the cause of the decline was a lessening of the secretion of prolactin to suboptimal levels The latter effect they differentiated from the " natural rate of decline due to the death or passing out of active service of the secretory cells '

The pointed relationship between the yield at the time of treatment and the response, even in animals all in comparable declining midlactation, again suggested an inherent or conditioned variation in prolactin secretion and supported the claims put forward by Asdell and associates Good yields from young virgin goats (58) after anterior pituitary injections are undoubtedly to be explained either by the presence before treatment of some degree of precocious mammary development—a comparatively frequent occurrence in this species—or by development resulting from gonadotrophic activity in the pituitary extracts used

De Fremery (95) and Trautmann and Kirchhof (277) have stressed the importance of some predevelopment of the gland before beginning treatment with lactogenic extracts if abundant secretion is to result These authors separately obtained good yields from virgin goats whose udders were first developed by administration of estrogens or whole ovarian extracts The first author believed that no milk could be obtained from such treated animals without further injections with prolactin preparations, and stated that "To start a lactation period an artificial increase of the prolactin (lactogen) level in the blood is therefore of importance ," a conclusion that, as we now know and as will be later explained, is only justifiable in a small proportion of cases

Parallel studies on the cow were introduced by Gruter and Stricker (120), who showed that, for cows already lactating, pituitary injections were able to increase the milk yields by very substantial amounts Stockklausner and Daum (267), who gave nine injections of a pituitary preparation over a period of 43 days confirmed these results using experimental and control groups of ten cows in different stages of lactation ranging from the fourth to the eleventh month after calving An application of the treatment to heifers (32) was as would of course be expected, less successful, but some milk was obtained its amount apparently

bearing a relationship to the extent of udder development already present when treatment was begun

More extensive trials involving 510 experimental animals and 90 controls were reported by Asimov and Krouze (10) Their animals were in various stages of lactation from the first to the twenty-first month after calving and were heterogeneous regarding breed, age, and breeding history These differences were balanced by corresponding variations in the control group The animals, experimental and control, were subdivided according to their original distribution in four milking sheds and the results, given as the total daily milk yield from these different subgroups, were expressed as a percentage of their corresponding daily yields during a preliminary period immediately preceding the experimental treatment In each group increases of 20–30% were found after single injections of an alkaline extract of ox anterior pituitary tissue, the effect was transitory, disappearing usually after six or seven days, when the lactation curves again approached those of the control animals Rather unexpectedly, in view of the reports of other workers, these authors obtained their greatest effects on milk yield in early lactation, their contention that crude anterior pituitary preparations were more effective than purified prolactin extracts is, however, in good accord with subsequent investigations (86,91) to be more fully appraised in a later section

Recent research on this problem of stimulating milk production in cattle has been largely confined to the work of Folley and Young, who have introduced certain new ideas, based on their experimental findings, which challenge the comparatively simple conception of the hypophyseal role in the control of milk secretion held previously by most workers Their views involve the appreciation of two distinct concepts—lactogenesis and galactopoiesis—both thought necessary for a full evaluation of pituitary function in the phenomenon of lactation (88) By lactogenesis they signify the initiation of lactation in a formed but nonlactating gland and deem this action to be mediated by the pituitary lactogenic hormone, probably in conjunction with other pituitary factors of which adrenocorticotrophin is almost certainly one and adrenal stimuli evoked by adrenocorticotrophin By galactopoiesis they refer to the ability—shown by a complex of pituitary principles, one member of which may be prolactin, but not as yet definitively described—to increase or maintain an already existing milk yield The distinction lies between the power to initiate and the power to perpetuate or augment Since their conclusions gained support from work already carried out on hypophysectomized animals it will be advantageous, before considering their views in full, to enumerate the effects produced by hypophysectomy

upon lactation and the results of attempts at replacement therapy using lactogenic hormone preparations, either alone or in conjunction with other trophic pituitary extracts

B Hypophysectomy and Lactation

The first report of direct significance in this field of inquiry was published by Allan and Wiles (3), who made the single observation that cats hypophysectomized in pregnancy made no attempt to suckle their kittens McPhail (172) confirmed this and gave some evidence that, after young had been aborted, the dry glands of the hypophysectomized cat could be stimulated to give some secretion by the injection of anterior pituitary extracts Further, McPhail reported two cases in which lactation had stopped following postparturient hypophysectomy These results in their broad implications are typical of many reports on other species, and we may cite here papers by Selye *et al* (254) and Newton and Richardson (204) on the mouse, by Pencharz and Long (209) and Selye and co-workers (255) dealing with the rat, by Nelson (192), Pencharz and Lyons (210), Macchiarulo (166), and Gomez and Turner (108) on the guinea pig, by Fredrikson (94) on the rabbit by McPhail (171) on the ferret, and by Houssay (134) and Lyons *et al* (162) on the dog The collective research of these workers leads to the general conclusion that hypophysectomy, if carried out during pregnancy, will prevent lactation, or if performed after parturition will stop it, the effects being independent of the amount of mammary glandular tissue present Further, it shows that the injection of suitable anterior pituitary extracts will permit, or restore, secretion in such operated animals The qualifications meriting the epithet "suitable' were and are still in some doubt, as will be shown below

Granted the pre-eminent role of pituitary prolactin in the successful control of lactation, experiments in which hypophysectomy was employed did indicate the possibility that other mechanisms have a special significance in the very earliest stages of lactation or even play a part in the normal maintenance of secretory function Thus, Selye *et al* (37,253, 254) gave evidence both for the rat and the mouse that the pregnant uterus and its contents could also be a source of secretory stimuli, albeit of a very temporary nature, at the time of parturition, animals completely hypophysectomized during the second half of lactation were observed to secrete milky fluids for a few hours after normal delivery, but the factors conducive to this secretion were in no way an alternative to the pituitary stimuli needed for the normal onset and continuation of milk production Newton and Richardson (204) have expressed the opinion that in the mouse the placenta can cause secretory activity

during the last week of pregnancy though the quantitative significance to its action might vary Such a mechanism might well explain the presence of milk in the glands of mice, hypophysectomized midway through pregnancy and killed after a further seven or eight days, before littering

The successful use of anterior pituitary extracts for remedying the inhibitory effects on lactation caused by hypophysectomy was naturally attributed to the presence of prolactin, and it was tentatively assumed that this specific hormone alone could be held responsible for such remedial action An account of experiments by Gomez and Turner (108) in which replacement therapy was attempted in female lactating guinea pigs hypophysectomized during the first week after delivery showed, however that whereas purified prolactin preparations were unable to maintain lactation ground aqueous suspensions of whole sheep pituitary tissue were most successful in keeping the gland in active secretion The first suspicion of a more complex pituitary control of lactation was thus aroused and earlier results with intact animals, having an endogenous hormonal secretion superimposed on any exogenous application, were seen to have been possibly misleading in their first interpretation

The problem of finding the additional pituitary principle or principles necessary for full replacement therapy was carried a stage toward its solution by Nelson and Gaunt (195), who first confirming the findings of Gomez and Turner extended them in a series of experiments showing that lactation could be induced in immature male and female guinea pigs hypophysectomized at the end of a period of gland-developing estrogen treatment provided that injection with prolactin was supplemented by the simultaneous administration of adrenocortical hormone extracts Similarly this combined treatment caused lactation in hypophysectomized pregnant females which failed to respond to treatment with prolactin alone Gomez and Turner (109) also demonstrated this active functional role of adrenocortical hormone preparations and further showed (110) that injections of the adrenocorticotrophic hormone prepared free from growth gonadotrophic, and lactogenic factors were equally effective when given in the same way—in conjunction with prolactin injections It was made clear in the foregoing reports that the adrenocortical hormone or adrenocorticotrophic preparations were unable to initiate or support lactation in hypophysectomized animals when given alone An attempt (211) to induce lactation in rats hypophysectomized on the day preceding delivery using similar methods, yielded results which, although partially successful, suggest that complete substitution therapy might be more difficult in this species It should be noted here that Fredrikson (94) obtained results from hypophysectomized, pregnant,

lactating, and pseudopregnant rabbits which were not in complete agreement with reports on the insufficiency of prolactin to maintain milk secretion His animals gave milk following injection of adrenocorticotrophin-free pituitary lactogenic extracts Possibly cortical hormone deficiencies are less speedily apparent in this species after hypophysectomy, though in one case milk was obtained even though the prolactin treatment was delayed until forty days after the operation

In explanation of all these results two theories could most readily be put forward, either the adrenal cortex has a direct functional role to play in the phenomenon of lactation, comparable with that postulated for prolactin, or its effect, as evinced in the hypophysectomized animal, is a concomitant of the more general metabolic disturbances, perhaps those of electrolyte and water balance, known to be associated with adrenal insufficiency, a view which gains some support from the successful use of salt therapy in the induction of lactation in hypophysectomized guinea pigs treated solely with purified prolactin (196) A full discussion of adrenal involvement will be given in a later section but here it may be pointed out that whether it proves to be direct or indirect in its action some cortical hormone, or possibly hormones would appear to be essential for the initiation of milk secretion and may be deemed to have "lactogenic" properties Folley and Young (89) in a discussion of the specificity or otherwise of the pituitary lactogenic principle state that "Since adrenotrophin as well as prolactin is necessary for the initiation of lactation in the hypophysectomized guinea-pig, there appears to be no useful purpose in maintaining the theory of the existence of a single lactogenic hormone, prolactin"

Emphasis has also been placed by some authors (see 112) on the important, if indirect, role played by those pituitary principles concerned in the regulation of carbohydrate levels in the blood It is claimed that any adverse effects upon carbohydrate metabolism resulting from their abnormal functioning may well be accompanied by serious fluctuations in milk yield, since glucose is such an important precursor of milk lactose

It will be clear that there are fine-drawn distinctions between the lactogenic attributes of the various pituitary and other principles found necessary for the initiation of lactation in hypophysectomized animals To enable normal milk secretion to start and to proceed is a property common to them all, yet this is achieved by a varying remoteness or indirectness of control, the nature of which can only be fully appreciated when the responses of the individual alveolar cell to hormonal stimuli are better understood In the control of secretory powers of such immense quantitative importance, the regulation, for example, of optimal cell membrane permeabilities or of gluconeogenesis such as may be

conceived to reside in the lactogenically active adrenocortical hormone must be considered every whit as important—even though of wider general significance—as the more specific secretion that we suppose pituitary prolactin to be

C Further Consideration of the Role of the Anterior Pituitary in Lactation

Reverting now to the experiments on intact farm animals carried out by Folley and Young (86) we may note that the major interest of their work in this field lies not in their confirmation of the work of Asimov and Krouze, already mentioned, regarding the augmenting effect of single injections of anterior pituitary extracts upon the milk yields of cows in declining lactation, but in their endeavor to correlate the galactopoietic activities of various extracts with their contents of different pituitary principles Their investigation gave unexpected results, for it was found that the degree of stimulation did not parallel the pigeon-crop gland-stimulating (prolactin) activities of the extracts used, but rather was closely allied to their glycotropic—"antiinsulin" (291)—potencies (see Table I)

TABLE I

Correlation of Galactopoietic Activities of Various Anterior Pituitary Extracts with Some of their Other Biological Properties (86)

Starting material	Extract	Pigeon-crop gland stimulating activity	Galactopoietic activity (single injection)	Glycotropic activity	Diabetogenic activity	Thyrotropic activity
Fresh ox anterior lobe	Crude saline extract	+++	+++	+++	+++	+++
	Prolactin C	++	+++	+++	0	Trace
	Thyrotropic-C	0	++	++	++	+++
Commercial dried ox anterior lobe	Prolactin 1	++	0?*	+	0	0
	Thyrotropic 1	0	+	+?	0	++

* No effect when a single injection was given Substantial effect when daily injections of a large dose were given over a period

A later paper (87) reporting the use of two of the extracts, prolactin-C and thyrotropic C, respectively *rich* and *poor* in pigeon-crop-gland-stimulating activity, to study the effects of repeated injections, showed similar results Both extracts caused marked increases in the milk yields, though as for single injections the effects were transitory and

disappeared quickly when injections ceased In these experiments the preparation rich in pituitary lactogenic hormone—*i e*, for our immediate reference, in pigeon crop gland activity—afforded the greater response, but this observation could also be correlated with the higher glycotropic activity of this extract

A still more complicated picture was presented by the results of a third study (88), in which a crude alkaline pituitary preparation, a prolactin preparation, and a glycotropic extract having a negligible prolactin content were injected on alternate days for a period of 22 days Contrary to the authors' earlier belief, the increase in milk yield did not in these animals parallel the glycotropic potencies of the extracts and only a slight improvement was observed in the yields from animals receiving the glycotropic preparation On the other hand, although the prolactin preparation did give an initial stimulus to milk secretion, the total effect during the injection period was only about one third that given by the crude pituitary extract, even though the prolactin unitage administered was roughly seven times as great

In view of this additional evidence on the need for postulating a more complex pituitary control of milk secretion, and bearing in mind the reiterated contention of Bergman *et al* (20) that the pigeon crop gland-stimulating factor is identical with the pituitary hormone initiating lactation in intact mammals, where adequate complementary adrenal stimuli may be presumed, the authors, as we have already noted, suggested that it might be desirable to distinguish between those substances responsible for initiating secretion and those which increase the yield from animals already lactating Bergman and Turner (21) have subsequently and independently put forward a similar suggestion claiming that " the anterior-pituitary secretes a number of hormones which individually and collectively have the ability to augment the lactation rate " They claim too that these hormones have no power to initiate lactation and believe that their galactopoietic action is due to an indirect and beneficial effect upon milk precursor levels They differ from Folley and Young in attributing the initiation of secretion to prolactin alone, and they further believe this hormone capable of producing galactopoietic effects on its own account when its rate of secretion is otherwise suboptimal in relation to the existing precursor level This postulate is not shared by Folley and Young, who reported the apparent destruction of galactopoietic powers by ethanol desiccation of pituitary material before its extraction (91)—a treatment known to be unharmful to prolactin itself They state that " prolactin in itself is probably not galactopoietic, though it may constitute one component of a galactopoietic complex '

Developing their work on the galactopoietic function of pituitary preparations, Folley and Young (91) obtained a sigmoid dose-response curve from cows following single injections of crude extracts of fresh ox anterior pituitary tissue, and increases of over 20% in the expected milk yields during the treatment period by injecting an amount of extract equivalent to 2 5 g of the fresh gland, on alternate days for three weeks It was also shown (62) that the galactopoietic effect was not exhibited at the peak of lactation, suggesting that at this period the galactopoietic hormone or hormone complex was not the limiting factor in milk production, and that extracts of pituitary tissue from different species showed very different galactopoietic powers (78) No information was given on the relative prolactin contents of the latter extracts, but it could be stated in respect to these experiments too that the augmenting effect was not correlated with the variations in prolactin content which might normally be anticipated in the species concerned (34)

Any attempted comprehensive account of the endocrinological control of milk secretion must necessarily be largely a matter of speculation at the present time, but it seems probable from the evidence we have at hand that the very complexity and multiplicity of the metabolic processes participating in normal milk production may call for an equally elaborate hormonal control Although it is not the purpose of this review to consider the biochemical aspects of lactation, for which reference may be made to recent accounts by Folley (67), Petersen (214), and Smith (259), it is necessary to emphasize that the mammary gland is the seat of many complicated syntheses and conversions, and that the plane of its biochemical activity, which in turn may be one determinant of milk yield, will be largely dependent upon the precursor levels supplied to the gland by the blood stream Thus agencies artificially lowering the blood sugar concentration have been shown to exert an adverse effect on milk yield (113,114) although the opposite effect of an increase in yield following an increase in the blood sugar concentration has not been conclusively demonstrated (25) We might therefore forecast that three distinct types of hormonal activity may play their part in determining the degree of lactation One of these, subserved by prolactin, we may suppose to be specific for mammary parenchyma and incidentally the pigeon crop gland epithelium, while the others are connected with more general phenomena and provide special aspects of membrane and water balance control—whereby the efficiency with which the gland can utilize a constant precursor concentration is maintained—and of the regulation of precursor concentrations, respectively

Any of these implicated hormonal mechanisms might thus prove a limiting factor at some stage of a lactation period, and possibly the rela-

tive hormonal adequacies during lactation may vary with the species For the cow it would seem that the factor which Folley and Young tentatively identify with the pituitary glycotropic hormone is the one most frequently present in suboptimal amounts, although, as has been observed, this is by no means always the case even in declining lactation The special case of the lack of any galactopoietic response to pituitary injections at the peak of lactation during the first seven weeks after calving presumably reflects a state in which all participating hormones are in adequate supply for most animals and the limiting factor is the inevitable and ultimate one of mammary development This view of multiple control and the conditioning of yields by individual limiting factors suggests further that to distinguish between those hormones able to initiate a lactation and those required for its maintenance may be artificial and unnecessary The distinction is seen to be apparent rather than real, the two aspects requiring identical control, but presenting phases of the lactation cycle in which different hormones are entailed as the limiting factor

The mechanism involved in the galactopoietic activity brought about by glycotropic extracts of pituitary tissue has no obvious explanation on the basis of the known properties of this hormone which does not, for instance, increase the arterial blood sugar concentration and cannot therefore permit an explanation on the grounds of an increase in precursor concentration, but an interesting suggestion arises from work of Jensen and Grattan (140), who identify glycotropic and adrenocorticotrophic hormones and thus provide a link between this galactopoietic function in normal animals and the stimuli necessary for initiating the secretory processes of hypophysectomized animals Grattan and Jensen have claimed (117) that glycotropic activity is only indirectly mediated by the pituitary, being in reality a property of certain adrenocortical hormones—corticosterone, 17-hydroxycorticosterone, and 17-hydroxy-11-dehydrocorticosterone, but not desoxycorticosterone This view is not, however, undisputed, and there remains a distinct possibility that the pituitary can elaborate a glycotropic hormone which acts independently of the adrenal glands (119,131,243) The implication of adrenocortical hormones in the problems of milk secretion will be further considered in the next section

II. The Adrenal Cortex and Lactation

Climenko and McChesney (36) have recently claimed an increased lactation in pseudopregnant rats ovariectomized prior to treatment with prolactin when adrenaline or cortical extract injections supplemented the treatment The augmentation with cortical extract appears to have

been rather dubious, however, and the results suggest that in these animals, with intact adrenal glands, the cortical hormone or hormones necessary for lactation were not limiting the response, or alternatively that the extract used was not rich in such principles The response with prolactin and adrenaline, however, possibly provides an interesting demonstration of the importance of precursors in determining mammary activity, on the warrantable assumption that it was directly dependent on an induced hyperglycemia, though the ability of adrenaline to stimulate cortical activity (283) presents an alternative explanation

Less equivocal results as to the part played by the cortical hormones have been gained by experiments involving adrenalectomy Carr (30) reported that rats adrenalectomized either one day before delivery or during the postpartum period when lactation was well established failed to maintain their litters and showed a premature drying off of the mammary glands at autopsy The injection of a cortical extract to similarly operated rats failed to act as a satisfactory replacement therapy (31) This failure of adrenalectomized animals to lactate or to perpetuate an established lactation has been confirmed by Gaunt (102), Britton and Kline (27), and others for the rat, and by Nelson and Gaunt (196) for the guinea pig, but the failure of replacement therapy with cortical extracts has not been a uniform observation Britton and Kline (27), for instance, were able to maintain the growth of litters suckled by adrenalectomized parturient rats given daily doses of whole adrenal extracts and Swingle and Pfiffner (270) claimed that adrenalectomized bitches given cortical extract would lactate normally after delivery It should also be noted that Cowie and Folley (42) have reported that adrenalectomy in their rats has caused a variable degree of inhibition of lactation in a number of separate experiments, the extent of the lactational failure—as judged by the "litter growth index" (41)—having a marked negative correlation with the intensity of lactation in members of simultaneously run control groups

This lack of agreement on the power of adrenocortical preparations to maintain lactation provided a background for the claim made by Brownell *et al* (28) of having demonstrated the existence of a specific cortical hormone, distinct from the life-maintaining hormone itself, which could support lactation To this substance they gave the name cortilactin indicating that lactogenic activity previously ascribed to the life-maintaining hormone was in fact due to this new substance If the existence of such an adrenal lactogenic hormone could be established, it is clear that a less confusing picture of earlier work would become theoretically possible, based on the probability of variations in the cortilactin contents of extracts used by different workers In a later paper Spoor *et al* (264)

have further investigated the properties of cortilactin extracts, and claim that the active principle, like prolactin, will stimulate the pigeon crop gland, though since Gaunt and Tobin (106) have shown that the latter hormone cannot stimulate lactation in adrenalectomized rats the two substances are thought to be quite unlike The authenticity of any adrenal hormone capable of stimulating the pigeon crop gland has been seriously called in question by Hurst *et al* (136), and at present cortilactin must be regarded as a doubtful entity

The value of salt therapy in counteracting to some extent the lactational sequelae of adrenalectomy has been stressed by Gaunt and Tobin (106), who found that successful lactation occurred in rats adrenalectomized before parturition and given either salt orally and cortical extract injections together, or large injections of the extract alone The authors were inclined to believe that the inadequacy of extracts, frequently reported, was really due to the inadequacy of the restorative dosages given to facilitate the supranormal fluid and electrolyte exchanges entailed in milk secretion An increased availability of salt on this theory may be supposed to have a sparing action on the amount of cortical hormone required The authors considered that their results offered no evidence in support of a second lactogenic hormone of the adrenal cortex, separate from the life-maintaining hormone, but on the other hand their work did not controvert such a view and the efficacy of large doses only of their extract, in excess of the amounts required to keep the rats alive and well, might be construed as the result of the presence of a specific lactation hormone contaminating in small amounts the life maintaining principle A confirmation of the importance of salt and water control in restoring lactation to adrenalectomized rats has been afforded by the histological study of Levenstein (151) Whereas salt therapy was seen to effect a large, but not complete, measure of repair of the mammary alveoli and preserved their function, water deprivation brought about changes in the normal lactating animal very similar to those seen in the glands of untreated adrenalectomized rats Levenstein considered that the changes attending adrenalectomy were not of a magnitude which would indicate the loss of a hormone specifically regulating mammary function, and adduced in support of this view, and in favor of secretory failure as the result of water imbalance, the fact that secretory activity does not cease immediately after operation In contrast to this result, Tobin (276) has concluded that salt therapy, although prolonging the survival of the adrenalectomized rats, has no effect on the survival of their litters, which, he found, died almost as quickly as the litters of operated rats given no treatment

It was clearly necessary, in order to gauge the relative importance, or

even to dogmatize on the existence, of salt effects and specific cortical lactation hormones in the processes of mammary secretion, that the research should be extended by the use, when they became available, of pure adrenal steroids in the place of the ill-defined extracts first employed "Cortical activity" has become a vague term, relevant to many biological manifestations, and in view of the many different steroids isolated from the gland it may well prove that control by the adrenal cortex is the resultant of the activities of numerous hormones, each having its own precise function, contributory to the central concept of "cortical activity' It might be hoped that the study, with pure substances, of such a clear-cut deficiency symptom as the failure of lactation in the adrenalectomized animal, would be of significance in research on the active steroid principles of the adrenal gland, as well as clarifying an aspect in the control of lactation hitherto very poorly understood Such an investigation has a greater interest because of the claim, already noted (28), that a distinct adrenal lactogen—cortilactin—does exist

The first attempts to maintain secretion in adrenalectomized animals using pure adrenal steroids were made with desoxycorticosterone Gaunt (103 104) found that lactation was not maintained in rats adrenalectomized within 24 hours of delivery and given daily doses of 0 1 to 1 0 mg of this hormone, although the mothers were free from any obvious symptoms of adrenal deficiency, and gained weight during the experiment In a later communication using the same substance, Gaunt *et al* (105) obtained results so variable that mean values were of little significance in judging the value of the treatment, some litters starving while others grew at a rate approaching that of normal animals in the same colony The authors likened their capricious results to those found when salt therapy is used and suggested that this particular steroid assisted lactation " only insofar as lactation is improved by the maintenance of general well being and health in the mother' Since the biological activity of desoxycorticosterone is bound up with sodium and chloride retention and potassium excretion, and not with carbohydrate metabolism, the analogy with salt therapy is probably apt and may deserve more extensive investigation The anomalous results obtained with this hormone have been further complicated by the work of Folley and Cowie (70), who found that, although unable to support a normal lactation, as shown by the suboptimal growth rates of the litters of treated adrenalectomized rats, desoxycorticosterone provided a better replacement therapy than other adrenal steroids which they used, and gave a survival rate of the young on the twenty first day after birth equal to 98% compared with a 59% survival in the case of the young of adrenalectomized controls receiving no injections (Fig 3) This work

has been confirmed and extended by these authors in a further paper (42) in which they have shown that the response to desoxycorticosterone is regularly related to the dose given, in any one experiment In separate experiments however with different groups of rats the response to a given dose may well vary widely, it is of particular interest to note that complete restoration of lactation was obtained in one of a number of groups given 3 mg desoxycorticosterone acetate daily—a result which, as the authors remark, gives further grounds for disputing the existence of a specific lactogenic hormone of the adrenal cortex

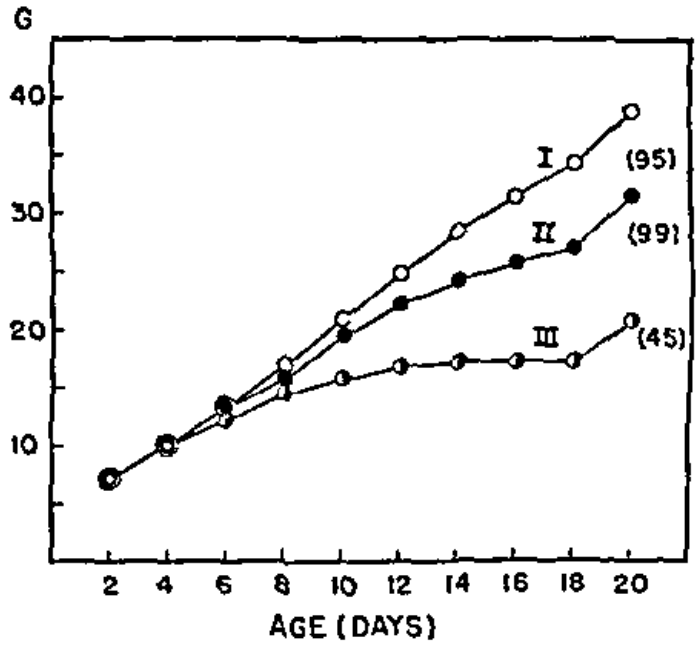


FIG 3 —Effects of adrenalectomy and of replacement therapy with desoxycorticosterone acetate on the growth rates of the litters of rats operated on the fourth day after parturition Curve I control litters Curve II litters of adrenalectomized rats given daily doses of 3 mg desoxycorticosterone acetate from day of operation Curve III litters of adrenalectomized rats Figures in brackets give percentage survival of young at twentieth day (From Folley and Cowie, 70 and Cowie and Folley, 42)

Evidence on the role of adrenal steroids in lactation has also been gained from hypophysectomized guinea pigs, for which Nelson *et al* (197) found desoxycorticosterone unable to replace cortical extract or adrenocorticotrophin in providing the adrenal stimulus, supplementary to prolactin, needed to ensure the successful initiation of milk secretion in such operated animals It was claimed that, far from promoting lactation, this steroid had marked inhibiting powers, normal intact animals prepared for lactation by previous estrogen injections failing to lactate as they would normally do when the estrogen stimulus was withdrawn, if desoxycorticosterone was given at this point, on stopping the

latter injections lactation began If, too, the desoxycorticosterone injections were supplemented with prolactin injections the milk flow was not suppressed, suggesting that the mechanism of inhibition operated on the production or release of prolactin from the hypophysis, an action considered in greater detail in Section IV, A (page 773), where the inhibitory properties of estrogens are discussed We may, however, mention here the relevant study of Turner and Meites (280) showing that desoxycorticosterone has no effect on the prolactin content of guinea pig pituitaries This inhibitory action of desoxycorticosterone on lactation in the guinea pig should be contrasted with the contrary reports on the absence of any inhibitory function of this substance on lactation in the rat (69), even when massive daily doses were given

The action of cortical extract or adrenocorticotrophin in assisting initiation of lactation by prolactin in hypophysectomized animals was however, paralleled, though to a smaller extent, by a second purified adrenal hormone, 17-hydroxy-11-dehydrocorticosterone (197) (compound E in Kendall s alphabetical designation) This substance had earlier (105) been reported to benefit the lactation responses of female rats, adrenalectomized immediately after parturition, when daily doses of 1 mg were administered, even to the extent of effecting a normal rate of growth in the suckling young up to the seventeenth day, at which time the survival was still 100% The same compound was also found to assist to some extent the lactation of adrenalectomized rats by Folley and Cowie (70), but for their rats the beneficial effect was not as great as that afforded by desoxycorticosterone

Finally a third adrenal steroid, 11-dehydrocorticosterone (compound A in Kendall's alphabetical designation), has been the subject of conflicting reports Gaunt *et al* (105) found that given to parturient adrenalectomized rats it allowed complete survival of litters up to the seventeenth day, though the weights of the young were slightly subnormal, while Folley and Cowie (70) reported definite deleterious effects on lactation as the result of replacement therapy with the same substance, adrenalectomized rats which received no treatment at all giving a better percentage survival of young at the twenty first day, and having an apparently superior milk flow for most of the experimental period The confirmation of their results with desoxycorticosterone in a later publication reporting different conditions of dosage and feeding has led Cowie and Folley (43) to the belief that, although the inhibition of lactation following adrenalectomy may not arise from the same primary cause in all cases, in their animals the customary partial breakdown of secretion could best be ascribed to interference with that mechanism—the electrolyte metabolism—with which desoxycorticosterone is particularly

concerned In this later work however these authors also observed slight beneficial effects on lactation following the injection of 11-dehydrocorticosterone in amounts smaller than they had previously used

The frequently confusing nature of the results attending the administration of pure adrenal steroids to adrenalectomized animals suggests that, as with the problem of ovarian hormones and mammary growth, we are here dealing with a complex ordering of hormonal influences which vary in their relative importance, both between different species and, more surprisingly, between any two strains of the same species So far any precise definition of adrenal involvement in lactation is beyond our power to give, but it seems probable that it is based upon the proper quantitative relationships between a constant number of adrenal principles, and that variations in response, such as have transpired in the work already reported, owe their incidence to variations from those quantitative hormonal ratios, secreted by intact or required by adrenalectomized females, which lead to optimal secretion in different individuals or species The full unravelling of the adrenocortical role is of course greatly complicated by the further probability that the active hormones owe their influence to general effects on carbohydrate metabolism or body tissues as a whole and not to any specific effects on mammary tissue in particular, for no evidence has yet appeared other than that given by Hartman and colleagues (28,264) to suggest the existence of a specific adrenal lactation hormone In their very uncertainty, those results already reported in which pure adrenal steroids have been used argue against any such powers for the substances investigated, rather, they appear to have their explanation in differential species and strain resistances to the interference with such systemic phenomena as electrolyte and water balance or to carbohydrate metabolism, which is inevitably involved in adrenalectomy experiments Replacement therapy on these considerations becomes a problem of rectifying these primary inadequacies, and varies in its success as the derangements vary in their relative, as well as their absolute, severities

III. The Thyroid and Lactation

Reference may be made to other reviews (*e g* , 217) for a survey of the many early, though rather sporadic, observations dealing with the thyroid gland and lactation Here it will be convenient to instance, as with a fair accuracy epitomizing these findings, the work of Graham (115) This author, testing the effects of both thyroidectomy and thyroid feeding on the milk yield of cows summarized his results as follows thyroidectomy had little or no significant effect on milk production—so far as yield was concerned—for, although there was a definite

decrease following the operation, a similar fall was noted in control animals subjected to sham operations only The addition of desiccated thyroid to the diets of normal or thyroidectomized cows, provided it was given in declining lactation and was not excessive in amount, caused definite increases in the yield His conclusion that any effect of the thyroids on milk secretion was dependent upon concomitant effects on the metabolic rates of the experimental animals, and not concerned with the basic hormonal control of lactation *per se*, has been endorsed and elaborated by later workers

Since Graham's report was published the effect of thyroidectomy upon lactation, particularly as observed in the rat, has been the subject of much contention Nelson and Tobin (203) found that rats thyroidectomized both during and prior to the onset of pregnancy suckled their litters normally, as evidenced by the equality of the growth rates of their young with those of litters of unoperated rats Similar results were also obtained with guinea pigs It was furthermore noted that the spontaneous lactation which occurs in guinea pigs on removal of a steadily maintained estrogen stimulus was not prevented by thyroidectomy, and that lactation could not be initiated, nor, where it had already started, could it be maintained, in hypophysectomized guinea pigs by injections of prolactin supplemented by desiccated thyroid given orally The latter result, which had been previously demonstrated and reported with other cognate observations by Gomez and Turner (111), offers a contrast to the successful lactation promoted under similar experimental conditions by treatment with prolactin and cortical extract, which has already been discussed The report as a whole indicated that the thyroid gland was not to be regarded essential for the processes of lactation, even though the possibility of its being able to condition the quantitative plane of secretion under certain conditions could not be prejudged Some of these experimental results were contested by Folley (66) with very clear demonstrations that lactation was subnormal in his rats, whether they were operated before or after parturition Even unilateral thyroidectomy, he found, caused an inferior growth rate in the litters of rats so treated, and incidentally, in his experience, thyroxine did not provide an adequate replacement therapy It might transpire, however, that the daily thyroxine doses given in his experiments (100 μg) were too great, Rowlands (242) having restored normal body growth in thyroidectomized immature rats with doses of a much smaller order (2 5 μg) The subsequent claim by Nelson (194) to have confirmed the findings of Nelson and Tobin on the normal growth rates of litters from thyroidectomized rats, the results of Preheim (216) in which only a slight decrease in milk secretion amounting to about 12% of the total normal supply was judged

to have followed thyroidectomy, and results presented by Karnofsky (143), substantially in agreement with the work of Folley (66), made the whole problem assume a formidable complexity

A clue to the variable factor underlying this lack of agreement is given by the report of Folley *et al* (79) on the role of the parathyroid glands in lactation. It should be understood that thyroidectomy in the rat inevitably involves the removal of all the parathyroid glands as well—though possibly some small traces of accessory tissue may be left in other sites—and, although in his earlier work on the thyroidectomized rat Folley (66) had found no alleviation of the inhibitory effect upon lactation when parathormone had been administered, the work was now repeated—with confirmatory results so far as the disastrous effect of thyroidectomy upon lactation was concerned—and partially successful substitution therapy was achieved by the use of autoplastic thyroid grafts which subsequent histological examination showed to contain parathyroid tissue Parathormone injections given to the rats after operation, in amounts much greater than had been used in the earlier experiment, also provided a definite, albeit incomplete, therapy Surprisingly perhaps, this action could not be enhanced by the simultaneous administration of thyroxine but again it seems likely that the thyroxine doses used were too high An examination of the tracheae, esophagi, and the surrounding muscular tissues of 29 thyroidectomized animals used in this study showed that five had unexcised accessory parathyroid tissue, and since this was so the possibility arose that the variations encountered in the results of different groups of workers might be due—in the light now thrown on the potential influence of the parathyroid gland on milk secretion—to variable amounts of this tissue left *in situ* in the operated animals Different operational techniques or the use of different strains of rat might well serve to make such an explanation justifiable, though of itself it will not of course reconcile the divergent views completely, for parathyroid therapy never promoted full, normal milk secretion Whether the failure to do this is to be attributed to the use of suboptimal or supraoptimal amounts of parathormone or to some other factor, such as the need for some measure of thyroid therapy as well, cannot as yet be stated; Karnofsky (143), for instance, has drawn attention to the importance of a satisfactory nutritional state in enabling thyroidectomized rats to suckle their young, while effects of the operation upon the qualitative composition of the milk itself are to be discussed in a later section Incidentally it may be observed here that, although our evidence on the role of the parathyroid gland in lactation is very scant indeed, there have been indications from other sources suggesting its participation in this process (29,35), and recently the first unequivocal

report on the effect of simple parathyroidectomy on lactation in the rat has been published by Cowie and Folley (40) Five of six rats so treated showed a marked decline in lactation—as judged by the growth rates and survival of their litters—while the sixth was unaffected and, in the absence of any more plausible explanation, may be assumed to have had more abundant accessory parathyroid tissue The inhibition of lactation in the five affected rats was almost as severe as that found in comparable thyro- parathyroidectomized animals

A recent report on the effects of thyroidectomy upon lactation in the bovine (263) has indicated that the operation causes a big reduction in total milk yield, which could be counteracted by oral administration of fresh thyroid tissue The absence of any control sham-operated animals in this experiment necessarily detracts from its significance in view of the earlier observations of Graham (115)

A far greater measure of unanimity has been realized concerning the effect of giving thyroid preparations to normal lactating animals, and a great deal of experimental work now testifies to the stimulating action of these substances on milk yield That the action is almost certainly due to the thyroid hormone, thyroxine, was first demonstrated by Graham (116), who found substantial increases in yield when this hormone was given to cows in declining lactation His results were confirmed by Jack and Bechdel (139), Folley and White (85), Herman *et al* (129), Hurst *et al* (137), Smith and Dastur (260), and Ralston *et al* (217), the last-named authors reproducing these results for the goat as well as for the cow As an example of the measure of the responses obtained we may quote the result of daily injections of 10 mg of thyroxine for fifteen consecutive days which, Folley and White (85) found, raised the total aggregate yield from four animals, treated during declining lactation, over the last seven days of their injection period 28% above the amount which might have been expected had not treatment been given (Fig 4)

Such results clearly were full of promise for the dairy industry and, in an attempt to overcome the difficulties presented by the hopelessly inadequate available quantities and the expense of naturally occurring biologically active material, or of synthetic thyroxine, recourse was made to the use of iodinated proteins—particularly iodocasein—which can be prepared cheaply in plentiful supply and show in varying degrees, by the oral route, the physiological properties associated with the thyroid hormone Reineke and Turner (234), Blaxter (22–24), van Landingham *et al* (148), and Reece (225) have separately demonstrated the stimulatory effects of such active proteins upon milk production For the bearing these investigations may have on practical problems of the dairy industry and for an estimate of the advantages and hazards which may

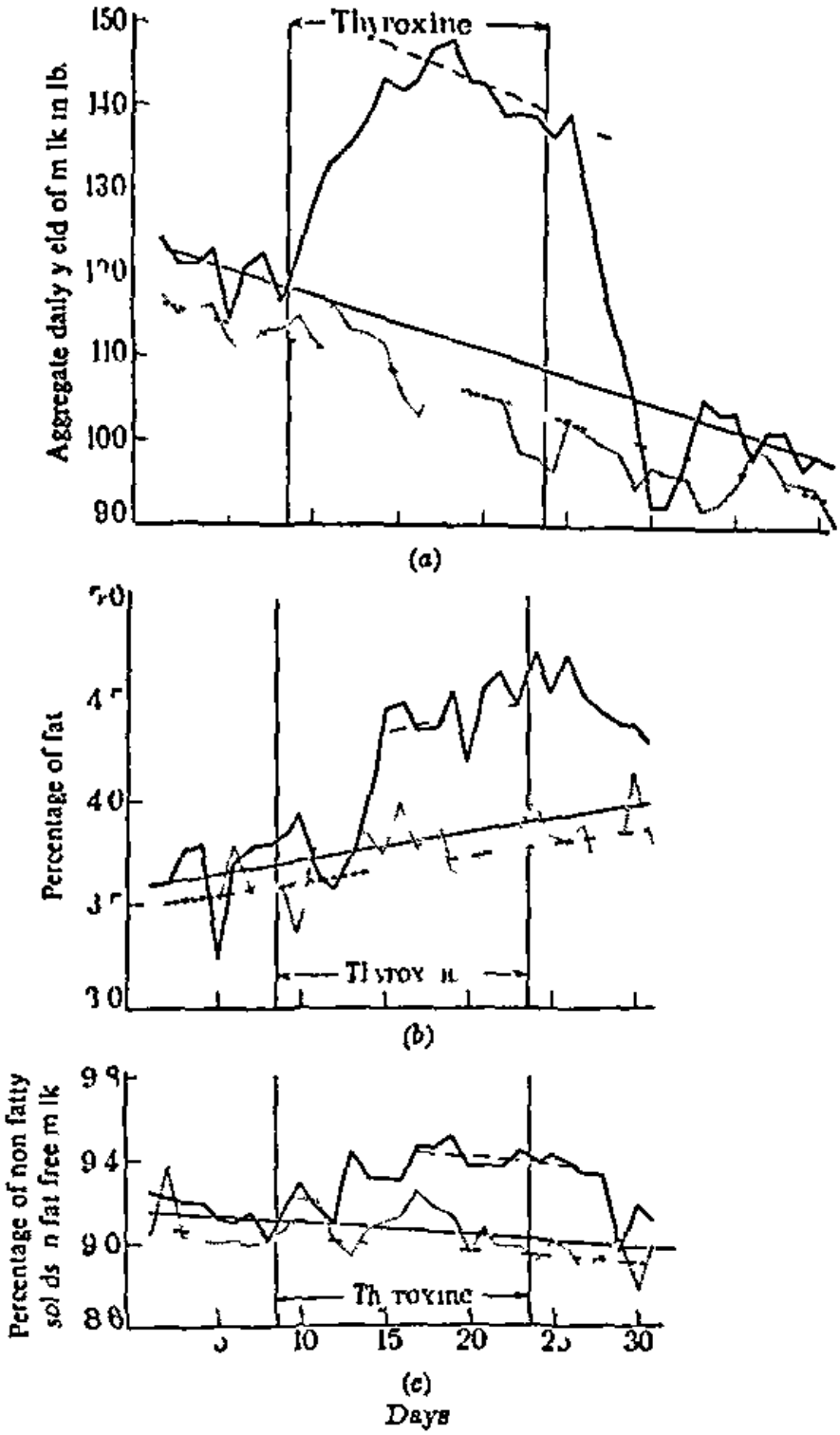


FIG 4.—(*a*) Aggregate daily yields of milk from thyroxine-injected and control groups of cows (*b*) Mean percentage of fat in milk secreted daily by thyroxine-injected and control groups of cows (*c*) Mean percentage of nonfatty solids in fat-free milk secreted daily by thyroxine-injected and control groups of cows ——injected controls — — — 28% 16% and 4% above basal values in (*a*) (*b*) and (*c*) respectively (From Folley and White 85)

be involved in any large scale and protracted use of this thyrogalactopoietic treatment the reader is referred to the extensive studies of Blaxter already quoted One or two points, however, are of immediate relevance in connection with our own discussion of the place of the thyroid gland in the complex control of lactation

The general consensus among those who have been working on this problem suggests that the degree of stimulation, whether by thyroid feeding thyroxine injection, or iodinated protein administration, is subject to wide individual variation and, although this fact alone cannot account for all the differences observed, it stands in some relation to the stage of lactation at which treatment is begun A greater effect is seen in declining lactation than at the peak or end of milk production Insofar as the first two of these phases are concerned Blaxter (22) has expressed succinctly the view that the effects indicate " a lower state of functional activity per unit of active mammary tissue during late lactation and a larger amount of active mammary tissue in a higher state of functional activity in early lactation " Thus, although some other reasoning will have to be adduced to explain the refractoriness of cows treated when approaching the end of their normal lactation period, the mechanism behind thyroid involvement in lactation is still believed primarily to be simply one expression of its general systemic influence on metabolic rates The production of a more quickly circulating blood stream a richer supply of milk precursors to the gland, and a heightened metabolic activity of the alveolar cells themselves are the most likely properties we can assign this hormone in its relation to milk secretion As lactation declines the plane of thyroid activity—as evidenced by other criteria—remains constant, and at no stage in the normal animal can an augmented yield be obtained by thyroid treatment without increasing to supranormal levels of activity other body functions such as respiration and the circulation of the blood The possibility of regarding the thyroid therefore as maintaining a level of activity for the mammary gland which even in the presence of a superabundance of all the other lactogenic and galactopoietic hormones, shall limit its functioning to a degree commensurate with the normal metabolic rates of other essential organs is attractive and harmonizes with the facts we have On this supposition thyroxine of course would never become a limiting factor in lactation in the normal animal, but is visualized as standing as the guardian of bodily resources so that they should not be expended by the additional, intrusive claims of milk production at a rate detrimental to the well being of the organism as a whole In contrast to the galactopoietic powers of pituitary extracts which might be regarded as a genuine resuscitation of waning mammary activity, the propensity of thyroxine

to stimulate milk secretion could perhaps be most faithfully represented as a fevered whipping of the whole organism into hyperactivity in the cause of benefiting one of its parts an over-all participation to produce a very local gain

The dependence of thyroid activity upon thyrotrophin secretion makes a knowledge of the variations undergone by this pituitary hormone during lactation of some interest Reece and Turner (232) have reported a higher pituitary content in beef as opposed to dairy cows, and Turner and Cupps (279) have presented evidence that in the rat pituitary thyrotrophin activity is higher during lactation than during pregnancy or the growth phase of the life cycle The absence of any lactogenic effect in pituitary extracts which could be ascribed confidently to their thyrotrophin contents was apparent from the work of Folley and Young already quoted (86), and unless new and contradictory evidence is brought forward we may assume that thyrotrophin is of importance in lactation only to the extent to which its influence gives rise to fluctuations in thyroxin secretion One anomalous report in which lactation in the guinea pig was diminished by thyrotrophin should be noted (121)

IV. The Ovarian Hormones and Lactation

A Induction and Inhibition

Recurrent reference has been made to the lactation induced in guinea pigs by the removal of an estrogenic mammary growth stimulus an effect which obviously suggests that estrogens should be regarded as exerting a suppressive action, preventing the onset of lactation in this species Much evidence has accumulated from other, and quite distinct experiments, reinforcing this view, and, although the effect of estrogens cannot be summarized in quite such simple terms as will be shown it is now generally conceded that one of the major effects of this group of hormones, whether naturally occurring or synthetic, can be a powerful inhibition of established lactation It would seem a logical step from this to the further conclusions that the initiation of milk secretion is also prevented during the gestation period by the high estrogen concentration known to be present in the body at this time, and that the sudden drop in the estrogen titer is sufficient to explain the rapid increase of secretion at parturition, such actions would clearly have a close resemblance to the estrogen administration and estrogen deprivation effects in the guinea pig which we have already mentioned, and which in fact, formed the basic tenets of what we may call the estrogen inhibition theory of lactation propounded by Nelson (193) to explain the sudden onset of copious lactation in parturient animals Nelson conceived the estrogens as

exerting their influence both by acting directly on the mammary gland itself, and by suppressing the secretion or release of prolactin However, certain other chains of evidence do not accord well with the view that estrogens, by themselves, hold the balance of secretion or nonsecretion in the developed gland, and counter-theories have been proposed which give them a less prominent place in this control

The reports of Parkes and Bellerby (207) and of de Jongh (141,142) showing that estrogen injections could inhibit lactation in parturient mice or rats, and a similar report by Smith and Smith (258) dealing with the rabbit, were the forerunners of numerous other papers presenting closely agreeing results Both Robson (239), using natural estrogens, and Noble (205), using diethylstilbestrol, demonstrated a dose-response relationship for the inhibitory effect, and showed also that if the injections were withheld lactation recommenced, indicating that within the limits imposed by their own experimental procedures no permanent damage was done to the secretory parenchyma This dependence of response upon the estrogen concentration—presumably the estrogen concentration in the blood would provide the most relevant figure—might explain the results of Folley and Kon (73) and Edelmann and Gaunt (53), who found the inhibition of lactation to be greater in the intact rat given estrogen injections than in the ovariectomized doe similarly treated Anselmino and Hoffmann (6) had earlier found no decrease in the growth rates of litters suckled by their injected ovariectomized rats and sought to explain this by reference to the well known action of estrogens in promoting secretion of the pituitary luteinizing hormone and so corpus luteum formation, suggesting that a product of this ovarian structure, not identified necessarily with progesterone, might be held responsible for the inhibitory effects in intact animals, rather than estrogen Since the other authors quoted got definite depression of secretion in ovariectomized rats, however, while Robson (239) and de Jongh (141) have reported complete suppression of lactation in ovariectomized mice, the theory seems quite inadequate, and as an alternative one might reasonably assume that in an intact animal the estrogen concentration, being derived from both endogenous and exogenous sources might, at any rate at the start of the injection period, be greater than that present in an ovariectomized but otherwise comparably treated animal, and that the differential effect observed could be due simply to differences in the concentration of this hormone Results quoted for the rat at the end of a paper by Walker and Stanley (285) are of interest in this connection It would seem likely that species differences might exist dependent upon two species variable factors the estrogen threshold for inhibitive effects, and the extragonadal estrogen

formation in the body The work of Folley and Kon (72) and of Folley (69), in which very large injections of progesterone were given to lactating rats without affecting their lactation, is of interest in this connection, but the results do not preclude the possibility that this hormone, although unable to inhibit lactation by itself, might have this power when in combination with some optimal concentration of estrogen

The interesting generalization, suggested by Folley and Kon (73) and further expounded by Folley (68), that power to inhibit lactation may be a property of substances able to cause mammary growth, particularly of the ducts, has perhaps not received the detailed investigation it deserves The species variations encountered in work on both these problems might, if reinvestigated under conditions eliminating other variables, show correlations of far-reaching significance We may instance testosterone, itself both a mammary growth stimulator and lactation inhibitor as many reports coming from fundamental research and clinical experience confirm (*e g* , 19,52,53,73,135,146,240), and androsterone, a substance unable to affect either mammary growth or lactation (73,240) as adding to the credibility of Folley and Kon's general thesis

Weichert and Kerrigan (288) have expressed the opinion that the young of estrogen-treated rats do not grow at a reduced rate because of failure of the mother's milk supply, but rather because the mother's solicitude for her young wanes and her maternal care becomes sporadic and dilatory Inadequate nutrition follows "a lack of opportunity to suckle rather than failure of the mammary glands to secrete" The authors based their argument on the presence of abundant secretion in the glands of treated rats whose litters were growing subnormally This explanation has enhanced interest in view of the demonstration by Bacsich and Folley (13) that in estrogen treated lactating rats, even when no suckling has taken place for some days because of the death of the young from inanition, the mammae show no signs of involution, the authors state " there was relatively little disintegration of the alveoli Some acini were distended with coagulated secretion "

The inhibition of lactation by estrogens has been confirmed for the cow (65,287), while numerous clinical reports have testified to a similar action in the human (*e g* , 14,92,189,218,286), where of course it is especially valuable as a method readily enabling the clinician to suppress secretion when for some reason breast feeding is not required or is undesirable Reports denying the power of estrogen, in this particular case diethylstilbestrol, to prevent lactation or to affect an established lactation, have been published by Abarbanel and Goodfriend (1) and Abarbanel and Klein (2), who attribute the inhibition noted by other workers

to the absence of the suckling stimulus, but in the light of so much contradictory evidence it is clear that these reports must be regarded with some scepticism, and the results themselves as atypical Even though cessation of suckling almost certainly is a contributory factor, it seems a matter of general clinical experience that inhibition by this means alone has nothing of the dramatic suddenness normally associated with estrogen treatment The authors' view that estrogens prevent painful engorgement of the breast by overcoming vascular and lymph stasis may well be true but it would seem probable that any relief so conferred would owe something to a similar action upon the flow of milk in the ducts

That estrogens in addition to their well attested inhibitory function should also under slightly different conditions—primarily we believe associated with dosage—be able to initiate milk secretion in nonlactating animals undoubtedly provides one of the most paradoxical problems that our subject presents The evidence for this derives almost entirely from experiments on farm animals where any significant changes following treatment may be directly measured and are of a quantitative character precluding doubt, sporadic references to a similar phenomenon occurring in small laboratory mammals have been made however (93, 153 223) A paper by Laqueur (149) deals with a comparable lactogenesis in virgin rats treated with testosterone propionate

It has already been observed that lactation can be induced in goats by dual treatment with estrogen as an agent of mammary growth, and prolactin as a specific for secretion (95,277) The first conclusive demonstration of copious lactation as the result of estrogen treatment alone was given later when Folley *et al* (80), inuncting the udders of virgin goats daily with an ointment containing 1% diethylstilbestrol dipropionate caused a flow of milk which at its maximum reached 1500 ml daily for one of the animals These results were independently confirmed by Lewis and Turner (152), who induced lactation in kids and nonpregnant goatlings following subcutaneous injection of diethylstilbestrol, but also found that similar treatment given to lactating goats was, on the contrary attended by a fall in yield The results of Folley *et al* (80) were fully recorded and extended in a second paper (83) in which the lactogenic action of estrogens was explained in terms of a stimulated output of prolactin in response to amounts of estrogen too small to cause inhibition The importance of dosage was clearly shown in a paper by Mixner *et al* (186) in which it was reported that while 0 25 mg diethylstilbestrol given daily by injection promoted lactation in nonparous goats amounts of 1 mg or more were inhibitory to animals already lactating

Parallel studies on cattle were first reported in 1940 and 1941 by

Walker and Stanley (284,285), who obtained very promising yields from two heifers, one gonadectomized and the other physiologically sterile, following repeated injections of diethylstilbestrol dipropionate either alone or in conjunction with testosterone propionate As in the case of the goat it was noticed that if the estrogen titer rose beyond a certain point inhibitory effects set in and the yield fell An independent account

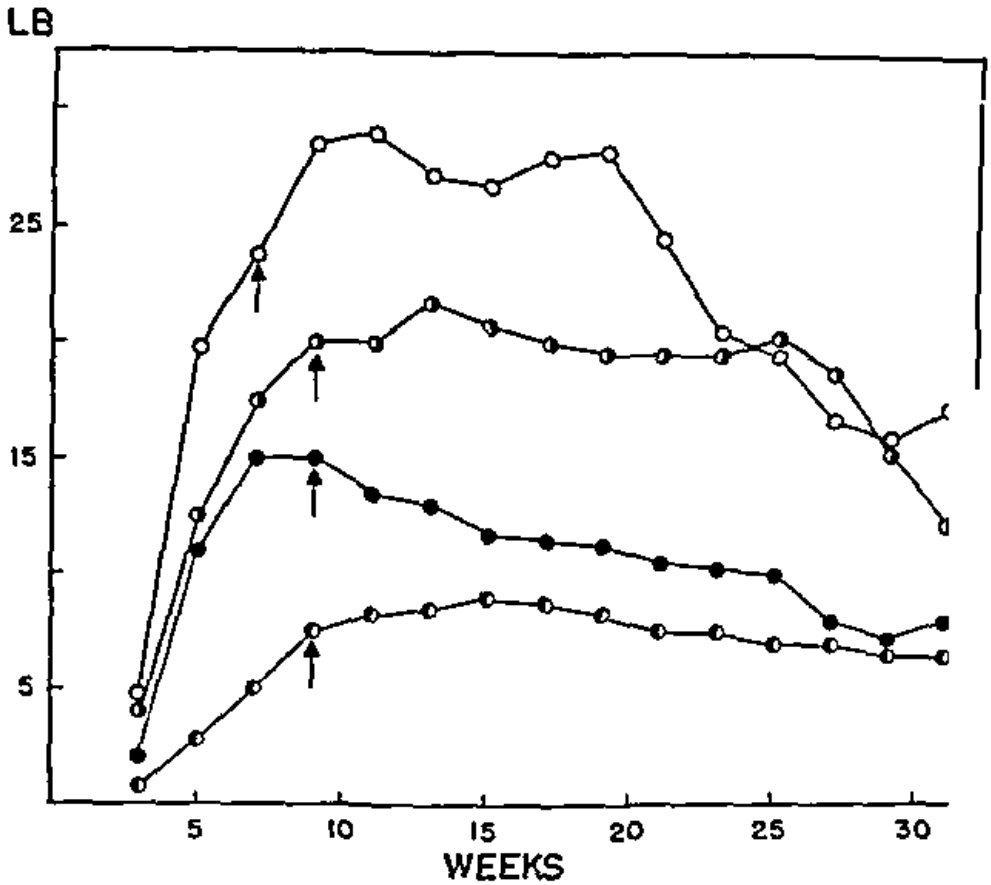


FIG 5.—Typical bovine lactation curves following treatment with synthetic estrogens by tablet implantation Abscissa time in weeks from start of treatment Ordinate yield in lb given as mean values over fortnightly periods Treatment stopped at arrows

of a less successful attempt to induce lactation in heifers by inunction of the udder region with 1% diethylstilbestrol ointment was also reported at this time by Folley *et al* (81), who, although only obtaining yields of the meager order of 100 ml daily, commented on the occurrence of regular fluctuations in yield correlated with the different stages of the estrous cycle These pioneer results on the artificial induction of lactation in dry, nonpregnant cattle by synthetic estrogens have since been very amply confirmed by workers using a variety of techniques for administering the estrogen single (208) or continued (224) injections of ester forms, subcutaneous implantation of tablets (74,84,127), and oral

administration (75) have been used, and in varying measure have shown themselves capable of promoting daily and total yields of the same order as those of normal lactation, and therefore of very great interest to the dairy industry (Fig 5) The original papers and one or two later, and for the most part purely supplementary, reports (49,265) should be consulted for an appraisal of the relative merits of these various procedures from the practical point of view Regarded as experiments having a bearing on our knowledge of the hormonal control of lactation they may safely be treated as a group, and there can be no doubt that each individual case, whatever the mode of treatment, gave or failed to give a successful response according to the degree in which the estrogen treatment elicited or failed to elicit certain endogenous hormonal stimuli providing a mechanism of lactogenesis common to them all It is by no means certain what hormonal mechanisms the induction of lactation by estrogens does involve, but certain general observations stand out prominently from the foregoing work which must very clearly affect any interpretation we may attempt to give, while the work as a whole has undeniable significance for any consideration of the hormonal forces operating to initiate lactation in the normal parturient animal Deferring for a moment a consideration of this last problem, we may summarize the pertinent points arising from the work on artificially induced lactation as follows

First, remembering the power of estrogens to inhibit as well as to promote secretion, comes the question of dosage It would seem a fair assessment of the work on this subject to postulate two distinct threshold values for estrogenic effects, a lower one, below which no changes except possibly some slight mammary development are observed, but above which lactation may be actively promoted *pari passu* with glandular development, and a higher one, above which lactation is inhibited and, conceivably, mammary growth too is affected adversely (see Chapter XV) This theory implies a range of estrogen concentration, derivable from endogenous or exogenous sources and lying between the two threshold values, which might usefully serve to promote the secretion of milk from existing glandular tissue, it constitutes an extension, for a particular case of the more general theory proposed by Folley (68) Folley and Malpress (74) published estrogen absorption figures for bovines implanted with estrogen tablets from which they concluded that a daily absorption of 12 mg was an amount which might be expected to give good lactogenic responses in this species, on the other hand an inspection of their figures indicates a very wide range of daily absorptions (3 7 to 23 9 mg) which, acting over comparable periods, evoked very substantial lactations, while yet again absorptions falling well within this range resulted in

negligible yields. A similar picture of great variations in response to estrogen doses of comparable size and duration can be gained from a study of the other papers on this subject already quoted

This individual variation forms the second major indication arising from the work on artificial lactation which has a bearing on fundamental theory. We may well suppose it to be due, according to the views already put forward, to differences in the threshold values for inhibitive and lactogenic effects among animals of the same species, differences which

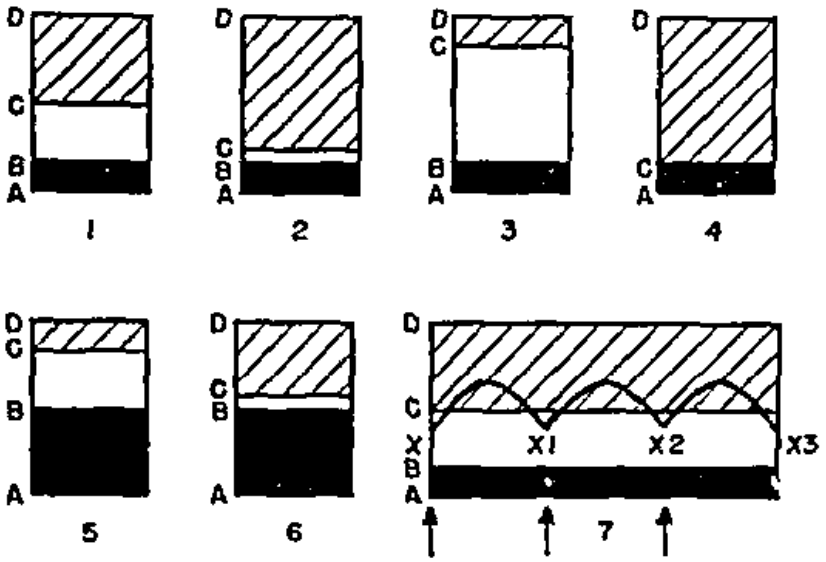


FIG 6.—Diagrammatic representation of the "double threshold" theory of estrogen activity in lactation, indicating how different responses might be obtained from animals having the same systemic estrogen concentration *Ordinates* systemic estrogen concentration, AB, range of estrogen inactivity, BC, range of estrogen lactogenic activity, CD, range of estrogen inhibitory activity $XX_1X_2X_3$ systemic estrogen concentration curve following estrogen injections at arrows

quite obviously might produce abnormally large or abnormally small interthreshold ranges depending on the sense and degree in which the thresholds themselves differ from mean values (see Fig. 6, Nos 1–6). Such intraspecies variations might no doubt be reproduced, and to a greater extent, in comparisons made among different species, since the prolactational estrogen range might theoretically be very wide indeed (Fig. 6, No. 3), in which case inhibition with physiological doses of estrogen would prove difficult, or at the other extreme be nonexistent (Fig 6, No. 4), in which case the inhibitive threshold would be judged to have fallen below a purely hypothetical and unrealizable stimulatory value.

The third widespread observation of importance in these experiments was the sudden jump in yield which often followed the cessation of

exogenous estrogen supply This suggests that, even when lactation is satisfactorily induced, the estrogen titer may eventually become too big and pass to the inhibitive range, unless suitable remissions of dose are periodically introduced

Granted the correctness of our postulates, the artificial induction of lactation by estrogens can now be visualized as a problem primarily of maintaining the estrogen concentration in the blood, or the body as a whole, within certain defined limits, which undoubtedly vary among animals and may vary for any one animal as its lactation proceeds or other physiological factors exert an influence The ability of dairy animals, for instance, to continue lactating even though a new pregnancy intervenes is perhaps an example in which a greater tolerance for estrogen exists due to the raising of the inhibition threshold under special circumstances

B The Initiation of Lactation

Some mention has already been made of Nelson's theory ascribing the suppression of lactation during pregnancy and its onset at parturition to, respectively, the high and rapidly falling estrogen titers present in the body at these times and to the differential effects of high and low estrogen titers on the secretion of prolactin The evidence adduced in favor of these views was extensively reviewed by Nelson in a series of publications (190,191,193), and it will suffice to say here that the theory commanded respect for the neat way in which it made use of the two separate experimental findings—the inhibitory powers of estrogens and the lactogenic properties of pituitary extracts—and sought to weld them into a constructive albeit tentative, relationship explaining lactogenesis at parturition The theory, however, requires a reassessment before it can be accepted as having validity at the present time, for it has been the subject of much criticism, and two of the major premises upon which it was built have been damagingly challenged

To take the less serious criticism first, we now know that estrogens are not purely inhibitory with regard to lactogenesis It would seem, however, that provided the theory is combined with the supplementary conception of threshold values outlined above it will still explain the remarkable lactational changes at parturition in as simple and plausible a manner as any hypothesis yet put forward In fact the theory gains, if anything, in the light of this recent evidence, since we may now assume that in passing at parturition from a high-concentration, inhibitory phase, the falling estrogen titers reach an actively lactogenic range in which production of prolactin and other lactogenic hormones is stimulated, a new and more positive expression thus being given to the original

and purely negative view that prolactin secretion follows passively the removal of an inhibiting agent The question of a direct inhibitory action of estrogens on the mammary gland itself remains a moot point, but it may be doubted whether evidence tendered in favor of this view, such as the inability of purified lactogenic extracts to promote lactation when injected into pregnant guinea pigs (190), necessarily requires this alternative explanation Indeed, in the same paper the author quotes experiments in which lactation in parturient guinea pigs was not stopped by estrogen injections provided a sufficient dose of prolactin was given at the same time, suggesting that, at any rate in the first instance, the action of estrogens is on the anterior pituitary

The most vulnerable part of Nelson s theory, however, was his assumption, based on cumulative but circumstantial evidence only, that the secretion of prolactin actually was suppressed by estrogens If this linchpin of his argument were to be proved unsound, the whole theory would become untenable and it is therefore with the reports treating with this aspect of the problem that we must now deal as possibly affording the most searching line of criticism directed at Nelson s original conjectures

The method usually employed for assaying the pituitary lactogenic hormone is the response of the pigeon crop gland to injections of the test substance or extract The method is considered more fully later, but here it is important to draw attention again to the fact that it is by no means sure that the pituitary hormone, or group of hormones responsible for initiating secretory processes in the mammal, is identical with the hormone—which we have throughout defined here as prolactin, or the pituitary lactogenic hormone—which causes development of the pigeon crop gland, in fact we have seen that there is reason to believe that the lactogenic "factor" may be an anterior pituitary hormone complex Current work which bases its argument on such an identity inevitably begs a question having the utmost relevance to its final conclusions, and, should only partial identity be established between the hormonal agents active in these two processes, an unconditional acceptance of assay values will inevitably be found to have prompted interpretations most misleading in their import when applied to problems of mammalian secretion The work of Folley and Young on the galactopoietic qualities of different pituitary fractions and the possible involvement of the glycotropic hormone in the lactogenic as well as the galactopoietic response, and the various reports of the inadequacy of purified pituitary lactogenic preparations to promote lactation in hypophysectomized animals even though they were able to stimulate the pigeon crop gland, are observations which, it will be recalled, have pointed to the need for a broader conception of pituitary lactogenic control, suggesting a complex mechanism in which

prolactin plays an important—perhaps the most important—integral part

Bergman *et al* (20) have attempted to throw some light on this rather confusing problem by assaying a pituitary lactogenic extract at different stages of purification by two distinct methods the pigeon crop gland response and the initiation of lactation in the glands of pseudopregnant rabbits Since the results from both methods changed in a similar way with purification, the authors concluded that the same hormone was accountable for the respective responses in bird and mammal This view was upheld in a later report (21) in which it was shown that, if a pituitary extract is subdivided into two fractions, the one rich in prolactin, and the other poor in prolactin but containing "thyrotropic and other hormones," the latter has practically no power to initiate lactation in pseudopregnant rabbits, intimating that this ability again is associated with prolactin content The argument is not entirely convincing however, for the authors found that the "thyrotropic and other hormone" fraction had a very pronounced power to supplement the rabbit lactogenic response to the prolactin fraction, enabling a given response to be obtained with smaller quantities of the latter than were required when it was used alone The interpretation that this was due to the power of the thyrotropic extract to affect favorably the levels of milk precursors seems to be begging the question, and in any case is no more justified than the conclusion that the extract contained an essential lactogenic component which was also present, though in suboptimal amounts, in the prolactin extract

However, Turner and his co-workers, from whose pens has come most of the criticism of Nelson's theory based on quantitative pituitary prolactin studies, have been satisfied to interpret pigeon crop gland assays as indicative of the total lactogenic potencies of their test pituitaries—or in other words, their power to initiate lactation, but the problem remains controversial, as may be the more readily judged by reference to a critical estimate of the position recently published by Folley and Young (89) Recognition of this fact will be made here by referring to the measurements of Turner, and others adopting similar experimental avian criteria as assays of prolactin only and not necessarily of the complete pituitary lactogenic mechanism

In 1937, Reece and Turner (232) collected much of their work on the prolactin contents of the pituitaries of various species in a comprehensive research bulletin In this, experiments were quoted leading, amongst others, to the following conclusions (1) no significant change takes place in the prolactin contents of rat or cattle pituitaries during pregnancy compared with the values given by nonpregnant animals, (2) for rats and

guinea pigs the content is increased during lactation, but for cattle the level is much the same as for dry cows, or during pregnancy, (3) ovariectomy decreases the hormone level, while injections of estrogen given to normal or ovariectomized rats or to normal guinea pigs have the opposite effect, and (4) a more tentative estimate that progesterone exerts no effect on the prolactin content of the glands of ovariectomized rats, untreated or given estrogen injections, when compared with suitable control animals

The first conclusion and the second, in the sense of an increase occurring after parturition, have since been confirmed with slight variations and the work has been extended to other animals (17,133,179,221,228), for the mouse rather higher relative levels of prolactin were encountered during pregnancy than in other species or in the mouse itself during nonpregnancy (138) The absence of lactation in the face of this increased pituitary prolactin content led Hurst and Turner also to conceive a doubt as to whether prolactin was the sole hormone concerned in lactogenesis It will be evident that the continuing low level of prolactin during pregnancy in most species and its sudden rise at parturition are in good agreement with the requirements of the Nelson theory provided increased pituitary prolactin signifies its increased production and not inhibition of its release, but it is equally apparent that the results afforded by estrogen injection and ovariectomy experiments are just the opposite of what the original theory demanded This work too however, has been confirmed (178,219) and Lewis and Turner have further shown that diethylstilbestrol when given to ovariectomized multiparous rats acts in the same way as the natural estrogens in augmenting the total pituitary prolactin content (154) It should be noted that such augmentation is normally the result of a dual response, an increase in pituitary size and an increase in the amount of prolactin per unit weight of glandular tissue, though it is not unusual for only one of these factors to be involved Finally, the progesterone effect has, since the first report, been expressed in a modified and more provocative form, for, although it was found (181) that this hormone itself had no direct influence on the prolactin content of immature female guinea pig pituitaries, it was observed that it could diminish substantially the increase in prolactin promoted in the same animals by estrogen injections

The last fact, together with the other data already given, led Meites and Turner to reject Nelson's theory and to propose an alternative which may be briefly summarized in the following terms during pregnancy the effect of a high estrogen titer on the prolactin content of the pituitary is nullified by the simultaneous presence of an active corpus luteum, or of corpora lutea, secreting progesterone At parturition the corpora lutea

regress and progesterone secretion stops, thus allowing estrogen to increase the prolactin output rapidly and with effect

The authors' full argument is contained in four papers (180–182,281) in which they summon further experimental evidence in support of their views Thus they were unable to show any diminution in the high prolactin content of the pituitaries of lactating rabbits allowed to become pregnant while suckling, and pointed out that according to the estrogen inhibition theory a fall in prolactin would clearly be expected in these circumstances (181) Further they demonstrated that, even when given in very high quantities, diethylstilbestrol was unable to produce any lowering of the prolactin concentrations in rat or guinea pig pituitaries in fact even with quite unphysiological concentrations of estrogen some increase in this value was always obtained Lastly, from their other investigations on this problem we may quote one claim of the very highest significance that the increase of pituitary prolactin in male rabbits following estrogen treatment has its parallel in a simultaneous increase in the blood prolactin, an observation of quite fundamental importance to the theory they have developed

Despite these extensive and objective studies upon which Meites and Turner have built their latest theory explaining the suppression of lactation during pregnancy and its onset at parturition, their claims are open to various criticisms which detract from its significance and hamper its acceptance as a worthy substitute for the original inhibition theory The points which might be raised have for the most part a wide reference and affect the interpretation of their results generally, but some are more specific and concern single experiments only It will be remembered that over all the work looms a doubt of the complete identity of prolactin with the pituitary lactogenic hormone or hormone complex, and hence the possibility that prolactin assays, taken alone, are irrelevant to the subject under discussion A second general criticism arises from the reflections of Hall and Nicolet (124) on the technique used by Turner and his co-workers in assaying the prolactin contents of their test pituitaries It was shown that the routine method, involving the local intradermal injection over the pigeon crop gland of suspensions of fresh or acetone-dried pituitary material (232) used in Turner's work, may give very erroneous and low results when compared with the values given by extracts of the same material tested by the same method Indeed it appears that the method may only measure 10–20% of the total prolactin content present in the suspended tissue It might be hoped that this would not affect the comparative values in any group of assays, even though absolute values might be at fault, but in a more detailed account of the earlier report, Hall (123) states alarmingly that " local tests

of suspensions are a good measure of pituitary tissue dosage but are almost useless as a test for total or relative prolactin content," and instances assays on two acetone-dried cattle pituitaries which, tested as extracts by the systemic injection method (122) or by the comparative local intradermal test, had potencies in the ratio 1 2, but when assayed as suspensions in the latter test had identical potencies

Thirdly, we have to consider the possibility that the inhibitory action of estrogen does not operate solely on the formation of prolactin in the pituitary, but on its release by the pituitary as well, and a satisfactory interpretation of most of the results of Meites and Turner can be founded on this supposition, linked always—in view of the dual and opposed roles which we recognize that estrogens subserve—with the conception of threshold values already proposed Thus the curve showing an optimum rise in the prolactin content of the pituitaries of small guinea pigs given different dosages of estrogen, reproduced here (Fig 7), might indicate that in the dosage range below that giving the peak prolactin value, the interthreshold phase of positive lactogenic stimulation and normal release is being traversed, while beyond this point the inhibitory zone is reached and the prolactin content falls precipitously Quite clearly, if release is hindered *pari passu* with the formation of prolactin, the pituitary content—in the absence of any intraglandular destruction—will never fall below the normal value, but will approach this figure more nearly as the dosage is increased and the rising estrogen titer in the animal's body takes less and less time to pass through the interthreshold prolactational phase before reaching an inhibitory concentration, this is exactly what Meites and Turner have observed It is important to notice that the fluctuating estrogen titers resulting from daily injections will in all probability allow long periods of lactogenesis even though the absolute amount of estrogen injected may theoretically be amply sufficient to cause inhibition (see Fig 6, No 7) Meites and Turner have in part anticipated these criticisms by their demonstration that the blood prolactin content of rabbits increases after estrogen administration (178) An inspection of their figures, however, shows that as the estrogen dose rises the blood prolactin increase falls and for the maximum dose used, 0 05 mg estrone daily for ten days—at which dose it would seem, by comparison with the published pituitary prolactin dose–response curve for guinea pigs and the few results given for the rabbit, that the inhibition threshold of estrogen activity might only just have been passed—the blood prolactin assay reveals little quantitative difference from that of a normal uninjected animal

Fourthly, some comment may be made upon the presumed action of progesterone in inhibiting the estrogenic effect on pituitary prolactin

concentrations Meites and Turner (181) showed that for immature female guinea pigs this action only occurred when the gravimetric ratio of progesterone to estrogen exceeded 250 1, approximately, and was only marked at much higher levels Even the threshold ratio differs widely from the ratio 40 1 which Scharf and Lyons (247) found necessary for

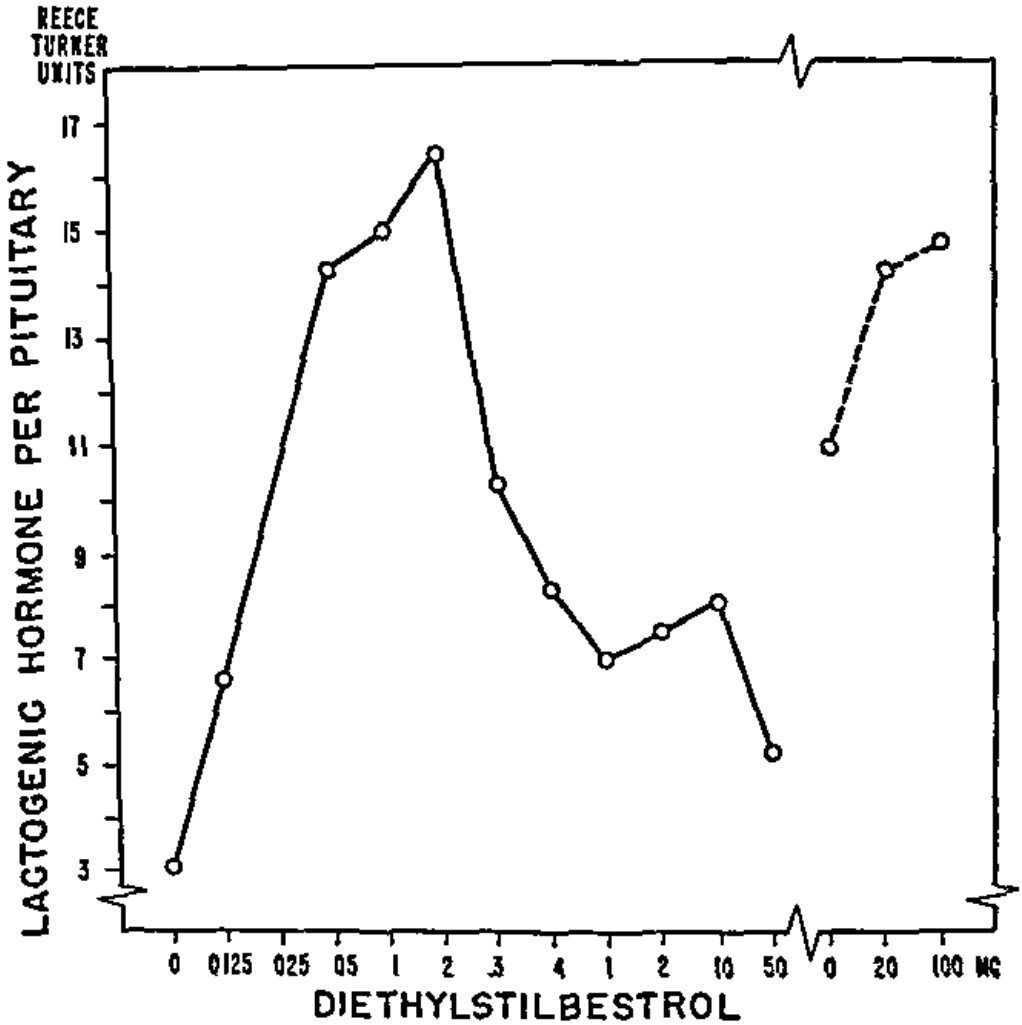


FIG 7 —Variations in the prolactin content of the pituitaries of guinea pigs given different doses of diethylstilbestrol (From Meites and Turner, 180)

o———o, small guinea pigs (av wt = 337 g)
o– – – –o, large guinea pigs (av wt = 509 g)

the satisfactory development of the mammary parenchyma in male rabbits, a ratio which, as we have shown in the preceding chapter, may have a more general application in problems of mammary growth A ratio of roughly 660 1 used in an experiment carried out by Lyons and McGinty (163) on a male rabbit resulted in very inadequate mammary growth It is possible, therefore, that the part which Meites and Turner would have progesterone play in antagonizing the effects of estrogen

during pregnancy may only be evinced under abnormal, unphysiological conditions and have no connection with hormonal relationships in the normal pregnant animal It is interesting to remark in this connection that the removal of rat ovaries which had been intensely luteinized by treatment with chorionic gonadotrophin (252) led to an abundant secretion of milk from developed glands An abnormally high progesterone estrogen ratio, which allowed the progesterone to override the effect of an otherwise prolactational estrogen concentration, may reasonably be assumed in these animals Other results of the effect of progesterone on pituitary prolactin contents have been reported by Reece and Bivins (227)

The observation that an intervening pregnancy has no effect on the high pituitary prolactin content of lactating rabbits (281) may be tentatively explained on the supposition that the estrogen inhibition threshold is raised under such exceptional conditions, a parallel is possibly afforded by the fact that, although estrogen values may be high during pregnancy, estrous or nymphomaniac symptoms are not customarily exhibited at these times It seems to us the more likely theory that when undue physiological strains have to be met, the organism should show some powers of accommodation modifying though only in a quantitative sense, the normal hormonal responses, rather than, as Turner and Meites suppose, that " factors present during pregnancy which inhibited lactogen (prolactin) production should inhibit (it) just as well during a period of simultaneous pregnancy and lactation " It should be remembered, too, that the presence of the placenta adds a complication to the picture of lactation control during pregnancy No clear evidence exists that this organ has any influence on the course of lactation in lactating pregnant animals and the fact that hysterectomy in the mouse at the eleventh day of pregnancy (26), or in the rat (250), is succeeded by lactation is probably a reflection of associated changes in the systemic estrogen value, rather than any more specific effect, such as the relief of uterine distension (118)

In concluding this criticism of the theory of Meites and Turner we note that, while they offer no rational explanation of the many instances, already quoted in this review, in which estrogens have been shown to stop milk secretion, they have proposed in order to reconcile similar findings of their own (180,186) to the tenets of their own theory, that either a decrease (180) or an increase (186) in the secretion of pituitary hormones, other than prolactin, may be held responsible In either case the effect is attributed to alterations in the availability of milk precursors, rather than to any specific action on the mammary gland itself We believe, however, that there are at present no valid grounds for discarding the

hypothesis that estrogens can depress the active systemic concentration of the pituitary lactogenic complex—using the term here in its widest sense—provided they are administered in concentrations above the inhibitory threshold The records of the beneficial action of pituitary extracts given to goats and cattle receiving doses of estrogen which were inimical to milk secretion (77,90,155) offer real support for this view Thus it would be difficult to explain, on the basis of precursor deficiencies alone, the need for anterior pituitary extract in order to *initiate* secretion in some of these estrogen treated animals, while in parenthesis we may add that the "progesterone-overriding effect" could scarcely be operative in such cases since the estrogen treatment has been shown to result in hypoplasia of the ovaries and functional inactivity (74,127) Folley *et al* (77) have ascribed this action of pituitary extracts entirely to their content of the lactogenic hormones, assuming that for such refractory beasts the estrogen was administered in quantities sufficient to inhibit the lactogenic action of the anterior pituitary and that secretion of these hormones was the limiting factor conditioning the absence of milk secretion Finally the galactopoietic effect observed by these authors and by Lewis and Turner (155), in goats or cows treated with pituitary extracts after the previous induction of lactation by estrogens, while most readily ascribed to the galactopoietic complex content of the extracts might also be explained in terms of lactogenic hormone administration The secretion of prolactin—which we may instance as one contributory factor to the total lactogenic effect—would probably be below maximal in most animals having interthreshold prolactational concentrations of systemic estrogen, and in consequence galactopoiesis would be expected to follow any treatment which could raise the systemic prolactin level Such an interpretation lays special emphasis on the authors opinion, already expressed, that lactogenesis and galactopoiesis may be different aspects of the activity of the same pituitary hormonal complex whose components vary in their relative importance both in the changing phases of the lactation cycle and, we might expect, under the sometimes artificial conditions of experimental procedure In the particular case we are considering the possibility that the beneficial effect of the pituitary extracts may have been attributable to prolactin—rather than glycotropin—clearly cannot be discounted by reference to the inability of prolactin to give a galactopoietic effect in normally lactating cows

The influence of hormones, other than those of the ovary, upon prolactin secretion has not yet received very extensive study, but Turner and Meites (280) have reported that injections of desoxycorticosterone acetate given to guinea pigs failed to influence the pituitary content of

this hormone, while it was believed (176) that the definite reduction which followed adrenalectomy in rats was probably due to secondary effects attending the operation, such as the cessation of estrous cycles, or a reduced food intake Since, however, the sudden rise of prolactin levels at parturition was demonstrated for rats adrenalectomized during the last week of pregnancy, it was further concluded that failure of lactation following this operation was not primarily a matter of prolactin deficiency

A fall in pituitary prolactin has been observed to accompany thyroparathyroidectomy in male rats (173), but in young goats thyroidectomy had no effect on the concentration of this hormone, which remained at the same level as in normal animals of comparable age (233)

In view of the common propensities of estrogens and the androgen testosterone to promote mammary growth and to inhibit lactation (73), it was obviously desirable to see whether the parallel extended further to a like effect on prolactin concentrations, and Reece and Mixner (229) have shown that injections of testosterone propionate into mature ovariectomized rats produced a 40% increase in pituitary prolactin content The additional fact that androsterone—which so far as is known has no mammogenic properties—has no power to affect prolactin levels (222) adds to the evidence suggesting a more fundamental connection than we may yet divine, between the mammogenic and lactogenic properties of substances known to be active in one or both of these processes

The prolactin content of pituitaries from rats given injections of pregnancy urine was no different from that of controls, while combined treatment with pregnancy urine and estrogen led to increased values, similar to those found in the pituitaries of rats given estrogen alone (226) The latter concordance, however, was not reproduced in the progress of lactation in the two groups concerned, and it was recorded that although pregnancy urine itself had no apparent effect on milk secretion, it did enhance the inhibitory action of estrogen given alone This supplementary inhibitory effect of chorionic gonadotrophin would seem therefore to be unassociated with prolactin secretion An increased mitotic activity in the mammary glands of the animals receiving the dual treatment prompted the authors to postulate a connection between the adverse effect on lactation and the additional impetus to growth given by the pregnancy urine This report on the action of chorionic gonadotrophin on lactation was preceded by a number of earlier observations of conflicting import (38,57,128), though in general inhibition has been demonstrated in intact animals The mechanism involved may be connected with the excessive luteinization produced in the ovaries by this

stimulus, and the presence of abnormal progesterone estrogen ratios in the body, but this has not yet been ascertained, alternatively the secretion of estrogen in response to the pregnancy urine may be implicated This possibility is rendered more likely by the observation that lactation is inhibited by both pregnancy urine and pregnant mares' serum in normal, but not in ovariectomized, rats (53) Increased luteinization could scarcely be postulated as a cause of the inhibition by the latter gonadotrophin The evidence, however, points clearly enough to some form of ovarian participation in these responses

Finally we may note reports on the effect of suckling on the prolactin content of pituitaries Selye and his colleagues (251,255,256) expressed the opinion that the production of prolactin might be to some extent under the control of nervous influences set in train by the nursing stimulus, and Meites and Turner (183) have since shown that the prolactin levels in the pituitaries of suckled postparturient rabbits are invariably higher than those of similar animals whose litters have been removed at birth Although, over long periods the suckling stimulus thus causes an enhanced production of prolactin, it would appear from results of Reece and Turner (230,231) that a depletion of the existing hormonal supplies is also entailed as a secondary—but usually masked—effect, presumably due to an accelerated release of the hormone One infers that the special conditions ruling in these last short-term experiments were such as to allow the rate of release of prolactin from the pituitary to exceed temporarily its rate of formation in response to the suckling stimuli applied, with a resultant fall in the pituitary prolactin contents of the suckled rats compared with their controls

V. The Pancreatic Islets and Lactation

Brief reference may be made here to the question of a possible interference with lactation following pancreatectomy, even in the presence of otherwise adequate insulin therapy It will be clear that even though no direct action of the pancreas upon normal lactation may be implied by the results of such surgical experiments, the essential part played by insulin in the intermediary metabolism of carbohydrates will inevitably confer upon this gland the power to influence lactation indirectly through precursor levels In accordance with the view that an indirect effect only exists, Markowitz and Simpson (168) and Cuthbert *et al* (45) have claimed that pancreatectomized bitches injected with insulin have been maintained through normal pregnancies and have subsequently lactated satisfactorily The subclinical glycosuria shown by these animals throughout the period of injection was markedly decreased during lactation and a transitory, but pronounced, hypoglycemia was reported by

Markowitz and Simpson in one animal, this effect can of course be readily explained by the need for glucose at this time for the synthesis of lactose Contrary evidence has been presented by Markowitz and Soskin (169), who found that two pancreatectomized bitches, maintained with insulin, which littered normally were unable to rear their pups, it seems possible—especially in the light of the interpretation given below of the later reports by Chaikoff and Lyons (33) and Nelson *et al* (198)—that, for some reason, *poor mammary development was the true cause of the inadequate* lactation

Chaikoff and Lyons (33), using depancreatized dogs kept alive by insulin, found that only one out of six operated animals was stimulated to lactation by injections of pituitary lactogenic hormone, although it was given in amounts more than sufficient to cause milk secretion in four unoperated animals Nelson *et al* (198), however, in similar experiments *found that lactation following prolactin injections was always obtained* in those animals having some measure of mammary development, and that, of their nine experimental bitches, the four which failed to lactate were those whose glands were involuted when examined prior to the initiation of treatment

From these results it would seem unwarranted to ascribe any specific lactogenic role to pancreatic secretions, but no final judgement can of course be made until experiments yielding more explicit quantitative data are reported

VI. The Posterior Pituitary and Lactation

Early work, associated with the names of Ott and Scott (206), Schäfer (244,245), Mackenzie (167), Schäfer and Mackenzie (246), Hammond (125), Turner and Slaughter (282), and Smith (261), concerning the relationship between the posterior pituitary gland and lactation has been recently reviewed by Folley (67) and by Petersen (212) As a result of these researches it *was evident that this gland*, while playing no direct part in the secretory activity of the alveolar cells, was important in the so-called "let-down" of milk, the latter term is in common use and recognizes the fact that the full milk yield from lactating animals can only be obtained when normal milking or nursing stimuli are applied Cannulation of the teat of the cow or goat for instance, will not give the maximal yield which, it seems from the researches of Maxwell and Rothera (170), Gaines (97), and Tgetgel (274,275), is dependent on an increase in the intraduct milk pressure—closely associated in practice with reflex stimuli arising from teat or nipple manipulation—whereby strong capillary forces present in the smaller milk channels being overcome, the milk is pressed out into the larger ducts, and

so to the milk cistern Writing in 1940, however, Folley was only able to relate these observations on the posterior pituitary gland to the normal milking or suckling processes in a noncommittal way, remarking that "There remains the interesting possibility, as yet unsupported by any experimental evidence, that the posterior lobe of the pituitary gland may play a part in the discharge of milk from the mammary gland during normal suckling" (67)

Some further advance has been made since this time, mainly due to the work of Ely and Petersen (55), who, by a suitable unilateral sympathectomy operation removed the source of efferent nervous stimuli to one half of the bovine udder This denervation had no effect on the processes of milk ejection, which could thus be confidently ascribed—as regards the terminal phase of its control—to hormonal influences in the normal animal, in support of this contention the authors were able to demonstrate an inhibition of "let down" in these animals following adrenaline injections and an equally rapid countering of this action in response to posterior pituitary extracts The theory was advanced that the normal release of milk is caused by afferent stimuli—not necessarily arising from the udder alone—causing the posterior lobe to secrete the oxytocic principle which in turn acts upon smooth muscle-like tissue (myoepithelium?) supposed to lie in close contact with the alveoli Evidence for the existence of such contractile elements has recently been presented by Swanson and Turner (269) The accordance between the predicted and observed results of several practical milking tests in which different manipulative techniques were used (185) strengthened the belief in the theory as illustrative of a fundamental mechanism in milking processes Petersen and Ludwick (215) have shown further that, if a surviving excised bovine udder is perfused with blood from a cow previously stimulated to "let down" her milk by appropriate handling an immediate flow of milk from the perfused gland results, while blood from unstimulated cows evokes no such response Both pitocin and pitressin are active in causing ejection of milk from the perfused bovine udder (213) or from the glands of lactating rabbits (278), the former being the more active However, although injection of these substances permits a more complete evacuation of the gland at a single milking it seems certain that their continued use over a period has no substantial *direct* effect on yield or fat secretion—that is, no galactopoietic effect (257), Knodt and Petersen (145) however, believe that it has an indirect influence and that a falling yield might be a result of the habitual retention of some milk in the gland due for example to faulty milking technique or to some idiosyncrasy of the cow They suggest that such incomplete ejection and its indirect depressive effect on milk production might be overcome by pitocin injections

An interesting report by Gomez (107) makes the claim, in harmony with the views of Petersen and his co-workers, that replacement therapy is greatly improved in hypophysectomized lactating rats given anterior pituitary extract, adrenal cortical extract and glucose, by the additional regular administration of posterior lobe extract

These views on hormonal mediation in that phase of lactation concerned with the release of the stored milk, call for the revision of the earlier concept of purely nervous control postulated by many authors, and in particular by Hammond (126), who suggested that the ejection of milk may best be regarded as an active process brought about by nervous reflexes, " causing erection of the udder and so pressure on the milk contained in the ducts," and gave incidentally a valuable appraisal of the earlier work pertinent to this subject

Dempsey and Uotila (50), Herold (130), and Desclin (51) have since given conflicting accounts of the effect of pituitary stalk section on lactation in the rat The two last-named authors found that rats so treated failed to lactate despite vigorous suckling stimuli, and concluded that nervous impulses essential to lactation pass to the pituitary via the pituitary stalk Dempsey and Uotila however claimed that any deficiency could be attributed to inadvertant damage of the anterior lobe and not to denervation, and concluded that stalk section does not interfere with normal lactation, a view which agrees well with reports by Smith (261) and Houssay (134) that lactation can take place successfully in the rat and bitch after the posterior pituitary has been removed

In view of the claims and counterclaims on fundamental issues and the demonstration by Selye and co-workers (see 251) that some measure of nervous control—"the suckling stimulus"—is implicated in the secretion of the lactogenic hormone from the anterior pituitary, it is not clear whether failure to lactate after stalk section is to be attributed to removal of afferent impulses to the posterior lobe, or is a reflection of deficient secretion of the hormones of the lactogenic complex resulting from suppression of stimuli to the anterior pituitary In any case, the need for further careful work in this field calls for no special emphasis Meanwhile the authors, from their critical but purely theoretical evaluation of the work bearing on this problem, are inclined to accept the views of Ely and Petersen, already considered, that the posterior pituitary gland is an essential factor in a neurohormonal arc controlling the "let-down" or "conditioned release" of milk already secreted and stored in the mammary tissues

VII. The Hormonal Control of Milk Composition

The various hormonal factors influencing lactation have so far been reviewed with regard to their power to alter milk yields, but the effect

these substances may have on the composition of the milk secreted is clearly of equal importance and may have far-reaching significance in questions of mammary gland metabolism Here we shall not deal with this biochemical aspect of hormonal intervention, merely reporting the compositional changes that have been recorded and noting that their source might be in still more fundamental changes in blood precursor levels, or in the specific action of hormones upon qualitative or quantitative aspects of mammary gland metabolism

Sykes *et al* (273) have reported that an anterior pituitary extract, given to cows in declining lactation for five-day periods, caused marked increases in both the total fat production and in the percentage of fat present in the milk This provided a confirmation of the earlier work by Folley and Young (87), who also recorded a marked rise in fat production with one particular extract, but were unable to correlate it with certainty to the presence of any particular hypophyseal hormone in their extracts A more recent report by Sykes *et al* (272) has shown that prolonged courses of injections with certain anterior pituitary extracts may lead, after the initial favorable effect, to adverse changes in milk composition, including dramatic falls in fat content It seems clear that the hormones present in the particular extract used in these experiments conditions the response, and the use of purer preparations, or at least the fuller characterization of extracts in terms of their hormonal contents, must be a *sine qua non* of further research in this field

The power of thyroxine to increase the total yield of milk fat, and also its percentage content, is well recognized, and the first detailed reports by Graham (115) have since been amply confirmed (85,129,217) Since the fat percentage is raised, the effect of thyroxine on this constituent must exceed that on yield itself and a differential action favorable to fat secretion must be envisaged Further evidence has more recently come from milk samples taken from cows fed iodinated proteins Reineke and Turner (234), van Landingham *et al* (148), and Reece (225) have reported very definite increases in this milk constituent in samples taken from treated goats or cows Smith and Dastur (260) have analyzed the milk fat given by cows injected with thyroxine and have found no important change in its chemical nature

Thyroidectomy performed on cows reduced the total fat yield by 75%, but this was due to the falling milk yield and not to any appreciable change in the percentage fat content of samples, which, like the nonfatty solids, remained unaffected (263), calcium and phosphorus contents of the milk were lowered by thyroidectomy in the goat (63)

The action of thyroxine on nonfatty solids secretion has been reported by some workers to be of small significance, but Folley and White (85)

and later, Blaxter (23,24) have demonstrated quite clearly that for cows, although relatively much smaller than the effect on fat secretion, a definite increase in the percentage of nonfatty solids is caused, provided the calculations are made on the basis of fat-free milk

The increase in the milk solids content, or "enrichment" effect of estrogens on lactation in the cow was first described by Folley (65), who later extended his first observations, made with natural estrogens, to similar experiments with diethylstilbestrol (82) With many of his animals the effect was complicated by concurrent falls in the volume of milk produced, but some showed clear-cut increases in both the total fat and total nonfatty solid values over long periods and in these cases a true galactopoiesis may be deemed to have occurred The "enrichment" effect has been confirmed by Spielman *et al* (262) The closely allied problem of the artificial induction of lactation by estrogens has led, from the practical point of view, to production of milk from both goats and cows of excellent chemical composition (76,83), the first milk secreted has invariably been of colostral type, but in those cases in which lactation has been successfully induced the composition has rapidly become normal with respect to all its major constituents

VIII Assay and Physiological Properties of Prolactin

As an addendum to this discussion of hormones and lactation it is proposed to give a brief account of recent work concerned with the assay and physiological responses to the pigeon-crop-gland-stimulating hormone—prolactin Work done to the end of the last decade has been authoritatively reviewed by Riddle and Bates (235), and the reader is referred to this excellent statement for a systematic review of the subject up to this time Problems of interest in the chemistry of prolactin and its preparation are considered in another chapter of this book, but reference may be made here to the reported detection of the hormone outside the pituitary gland itself—notably in urine (164,177), blood (150), liver (44), and placenta (54) Inconclusive evidence, which points to the eosinophil cells of the pituitary as the source of the hormone has been presented by Azimov and Altman (12), Schooley and Riddle (248) and Friedman and Hall (96)

Of the two general methods used for quantitative assay, the stimulation of the crop gland in pigeons or doves and the induction of lactation in the pseudopregnant rabbit or the hysterectomized guinea pig, the former has been overwhelmingly the most popular, for, although bearing a less obvious relationship to problems of mammalian lactation and yielding assay values which only with important reservations can be proffered as a measure of the mammalian lactogenic hormone complex,

in most of its forms it has a more objective basis, is simpler in execution, and is independent of other subsidiary or predisposing hormone mechanisms The method has, however, a number of variants, and results obtained using one technique have not been readily translatable into the unitage given by another, the need being manifest for a standard preparation of prolactin which should be internationally recognized and used in comparative work This need has now been met and an international unit defined as "the specific activity contained in 0 1 milligram (=100 gamma) of the standard preparation" Comparative assays with this standard and results expressed in this unitage are only accepted as valid when tests " which depend on the measurement or observation of growth produced in the crop gland of the pigeon or dove, whether as the result of systemic or of local administration" are used (56,184)

The variants of the crop gland test may be subdivided into (*1*) tests which depend on the determination of weight changes in the stimulated glands as the result of systemic injections, as originally proposed by Riddle *et al* (237), (*2*) tests which depend on a minimal stimulation of the crop gland following systemic injections (174), and (*3*) those involving a minimal or otherwise defined, stimulation after local intradermal injections immediately over the crop gland, as first suggested by Lyons and Page (164) and modified by Reece and Turner (232) The method of choice is largely a matter of the personal preference of the worker concerned except that the last may be used to detect far smaller amounts of the hormone, and also lends itself to comparative studies on the same bird since within limits the injection stimulates only that gland immediately beneath the injection site, leaving the other free either as an untreated control gland or, for example, for simultaneous proliferation by a standard prolactin preparation

The value of the results obtained by these tests depends on a rigid standardization of procedure for any comparative series, since factors such as the route and volume of the injections and the age and strain of the birds used may influence the response in greater or less degree A consideration of such variables, of their effect on response, of the experimental indications which bear upon their minimization, and the construction of suitable dose–response curves for different experimental conditions has continued to engage the attention of workers in this field, as comprehensive studies by Folley *et al* (71) and Hall (122) on the crop weight assay, and by Hall (123) on the local, intradermal injection test bear witness Supplementary studies on individual factors affecting the crop weight response have been made by Bates *et al* (18) on the use of different races or strains of doves and pigeons, by Bates and Riddle (16) on seasonal variations in response, and by Friedman and Hall (96)

on the influence of contaminating inert material in the extracts tested Bates and Riddle (15) have also investigated the effect of altering the volume of the injected extract in the local intradermal test, and found that great variations of response could be caused thereby, but this has been disputed by Meites *et al* (175) Finally Lahr *et al* (147) have reported that various substances of both biological and nonbiological origin have the power of producing nonspecific stimulation of the crop gland in the local test, though the concentrations required are not such as to prejudice the value of the method as effectively specific for prolactin The authors do point out, however, that the extreme sensitivity of this test may owe something to the fact that wound effects incidental to the intradermal injection may fall only slightly below the threshold for a positive response

Bergman *et al* (20) have reported a comparative study in which a quantitative relationship was sought between the units in which the results of various kinds of pigeon assays and of the pseudopregnant rabbit assay have in the past been expressed Their findings were related to the international unit in a later publication (175), when more particularly the amounts of the international standard preparation required to give minimal stimulation of the crop gland under defined conditions, by subcutaneous, shallow intrapectoral and local intradermal injections, were determined They were, respectively, 1 0, 1 25, and 0 006 I U Lyons (158) too has given results obtained with the international standard preparation, in which it was compared with other preparations of greater potency An account of an assay based on the initiation of lactation in the virgin guinea pig is also given, and it is noted that the relative effective amounts of the preparations used differed for the guinea pig and pigeon crop gland tests, a finding which vindicates the limitations attached to the use of the international unit

In their general account of prolactin, Riddle and Bates (235) consider the physiological properties of this hormone under a variety of headings lactation, crop sac stimulation, the ability to promote an exhibition of the instinct of maternal behavior in rats or of broodiness in fowls, the power to affect adversely the normal activity of the testis and ovary ("anti gonad" action), the effect on basal metabolic rate in thyroidectomized pigeons (calorigenic action), the promotion of body growth, splanchnomegaly, and effect on carbohydrate metabolism There is now ample evidence that a further property must be added to this list—that of maintaining functional activity of the corpus luteum This may prove to be related to the "anti-gonad" effect mentioned above

Astwood (11) showed that certain pituitary extracts were "luteotrophic," permitting the corpora lutea of normal estrogen-treated rats

and of hypophysectomized rats to continue functioning, as evidenced by the persistence of vaginal mucification He considered the luteotrophic hormone to be allied in its physical characteristics with lactogenic and adrenocorticotrophic, hormones, it now seems highly probable that the action he observed can be directly related—insofar as the underlying hormonal mechanisms are concerned—to the production of traumatic placentoma in rats whose lutein tissue has been activated to the secretion of progesterone by pituitary lactogenic hormone injections (59,60) Cutuly (46–48) has swelled the evidence pointing to a luteotrophic action of prolactin by maintaining pregnancy with this hormone in rats mated and then hypophysectomized either before or after implantation of the ovum, some reason for believing that this action is not entirely specific for prolactin, but may be shared, in part at least, by the gonadotrophic hormones of the pituitary, was also given by this author The contention, so far as prolactin is concerned, however, has been widely upheld by later workers (61,64,132,156,165,201,271) some of them using different criteria of luteal activity, for example mammary growth (101,165) It provides an interesting speculation that the absence of copious lactation during pregnancy, even in the presence of developed mammary tissue and a moderate amount of pituitary prolactin, may owe something to the need for this hormone to act as a luteotrophic agent during this period, an action which, as has been indicated earlier in this chapter, also implies a very essential role for prolactin in the processes of mammary growth

This and the previous chapter on the mammary gland were completed in February, 1946 No work has been published in the intervening two years which necessitates any serious revision of the concepts discussed in either chapter, unless we except the contention of Fauvet (*e g*, *Arch Gynakol* **171**, 342 1941) that estrogens in nontoxic doses do not inhibit lactation in the rat save in the presence of progesterone (see also Barsantini and Masson, *Endocrinology* **41**, 299, 1947) Nevertheless we think it desirable to refer readers who may be interested in citations of some relevant papers which have been published or have come to hand since the present contributions were prepared, to a Symposium of articles on Lactation which has been published in a recent issue of The British Medical Bulletin

The Symposium contains reviews on structural features of mammary tissues by K. C Richardson (*Brit Med Bull* **5**, 123, 1947), on the endocrine control of mammary development (*Brit Med Bull* **5**, 130 1947) and of lactation (*Brit Med Bull* **5**, 135, 1947) by S J Folley, on experimental galactopoiesis by F G Young (*Brit Med Bull* **5**, 155, 1947) and on the experimental induction of lactation by F H Malpress (*Brit Med Bull* **5**, 161, 1947) In addition mention should be made of the inclusion of a review on the nervous system and lactation by S J Folley (*Brit Med Bull* **5**, 142, 1947), since, as far as we are aware, this is the only extensive contemporary review of this aspect of lactational physiology which has so far appeared It is relevant to the topics discussed in the present contributions because it mainly deals, in greater detail than was possible in this book, with neurohormonal mechanisms involving the evocation, by suckling or nursing stimuli, of the secretion of anterior pituitary hormones

concerned in lactogenesis and galactopoiesis on the one hand, and on the other with the postulated involvement of posterior lobe factors in the mechanism governing the expulsion of milk from the alveolar tissues (and its subsequent withdrawal from the mammary gland) at milking time

REFERENCES

1 Abarbanel, A. R., and Goodfriend, M J *Am J Obstet Gynecol* 40, 1037 (1940)
2 Abarbanel, A. R , and Klein, M *D N Y State J Med* 41, 383 (1941)
3 Allan, H , and Wiles, P *J Physiol.* 75, 23 (1932)
4 Allen, E , Gardner, W U , and Diddle, A W *Endocrinology* 19, 305 (1935)
5 Anselmino, K J , Herold, L., and Hoffmann, F *Zentr Gynäkol* 59, 963 (1935)
6. Anselmino, K J , and Hoffmann, F *ibid* 60, 501 (1936)
7 Asdell, S A *Cornell Vet.* 21, 147 (1931)
8. Asdell, S A *Am. J Physiol* 100, 137 (1932)
9 Asdell, S A , Brooks, H J , Salisbury, G W and Seidenstein H R *Cornell Univ Agr Expt Sta Mem. No.* 198, (1936)
10 Asimov, G J , and Krouze N K *J Dairy Sci* 20, 289 (1937)
11 Astwood E B *Endocrinology* 28, 309 (1941)
12 Azimov, G I , and Altman, A D *Compt. rend acad sci U R S.S* 20, 621 (1938)
13. Baesich, P , and Folley, S. J *J Anat* 73, 432 (1939)
14 Barnes, J *Brit Med J* ii, 601 (1942)
15 Bates, R. W , and Riddle, O *Proc Soc Exptl Biol Med* 44, 505 (1940)
16 Bates, R. W , and Riddle, O *J Biol Chem* 140, cxlii (1941)
17 Bates, R. W , Riddle, O , and Lahr, E L *Am J Physiol* 113, 259 (1935)
18 Bates, R. W , Riddle, O , and Lahr, E L. *ibid* 125, 722 (1939)
19 Beilly, J S , and Solomon, S *Endocrinology* 26, 236 (1940)
20 Bergman, A J , Meites, J , and Turner, C W *ibid* 26, 716 (1940)
21 Bergman, A J , and Turner, C. W *J Dairy Sci* 23, 1229 (1940)
22 Blaxter, K L. *Nature* 152, 751 (1943)
23 Blaxter, K L. *J Endocrinol* 4, 237 (1945)
24 Blaxter, K L. *ibid* 4, 266 (1945)
25. Bottomley, A. C., Folley, S. J , Walker, F H A , and Scott Watson, H M *ibid* 1, 287 (1939)
26 Bradbury, J T *Proc. Soc. Exptl Biol Med* 30, 212 (1932–1933)
27 Britton, S W , and Kline, R. F *Am J Physiol* 115, 627 (1936)
28. Brownell, K A , Lockwood, J E , and Hartman, F A *Proc Soc Exptl. Biol Med* 30, 783 (1932–1933)
29 Campbell, I L. *J Dairy Sci* 25, 708 (1942)
30 Carr, J L *Proc Soc Exptl Biol Med* 29, 128 (1931–1932)
31 Carr, J L. *ibid* 29, 131 (1931–1932)
32 Catchpole, H R., Lyons, W R., and Regan, W M *ibid* 31, 301 (1933–1934)
33 Chaikoff, I L., and Lyons, W R. *Am J Physiol* 106, 716 (1933)
34 Chance, M R A., Rowlands, I W , and Young F G *J Endocrinol* 1, 239 (1939)
35 Chandler, S B *Anat Record* 53, 105 (1932)
36 Chmenko, D R., and McChesney, E W *Endocrinology* 28, 710 (1941)
37 Collip, J B , Selye, H , and Thomson, D L. *Proc Soc Exptl Biol. Med* 30, 913 (1932 1933)
38 Connon, F E *ibid* 37, 52 (1937–1938)

39 Corner, G W *Am J Physiol* **95**, 43 (1930)
40 Cowie, A T, and Folley, S J *Nature* **156**, 719 (1945)
41 Cowie, A T, and Folley, S J *J Endocrinol* **5**, 9 (1947)
42 Cowie, A T, and Folley, S J *ibid* **5**, 14 (1947)
43 Cowie, A T, and Folley, S J *ibid* **5**, 24 (1947)
44 Cunningham, B, Bickell, E A, and Tanner, M *Anat Record* **78** (suppl), 87 (1940)
45 Cuthbert, F P, Ivy, A C, Isaacs, B L, and Gray, J *Am J Physiol* **115**, 480 (1936)
46 Cutuly, E *Proc Soc Exptl Biol Med* **47**, 126 (1941)
47 Cutuly, E *ibid* **48**, 315 (1941)
48 Cutuly, E *Endocrinology* **31**, 13 (1942)
49 Day, F T, and Hammond J, Jr *J Agr Sci* **35**, 150 (1945)
50 Dempsey E W, and Uotila, U U *Endocrinology* **27**, 573 (1940)
51 Desclin, L *Compt rend soc biol* **134**, 267 (1940)
52 Duffy, P V and Corsaro, J *J Am Med Assoc* **116**, 33 (1941)
53 Edelmann A, and Gaunt, R *Physiol Zool* **14**, 373 (1941)
54 Ehrhardt K *Münch med Wochschr* **29**, 1163 (1936)
55 Ely F and Petersen, W E *J Dairy Sci* **24**, 211 (1941)
56 Emmens, C W *Quart Bull Health Organization League Nations* **8**, 901 (1939)
57 Enzmann, E V, and Pincus, G *Am J Physiol* **103**, 30 (1933)
58 Evans, E I *Proc Soc Exptl Biol Med* **30**, 1372 (1932–1933)
59 Evans H M, Simpson, M E, and Lyons, W R *ibid* **46**, 586 (1941)
60 Evans, H M, Simpson, M E, Lyons, W R, and Turpeinen K *Endocrinology* **28**, 933 (1941)
61 Everett, J W *Endocrinology* **35**, 507 (1944)
62 Fawns H T, Folley, S J, and Young F G *J Endocrinol* **4**, 205 (1945)
63 von Fellenberg, T, and Gruter, F *Biochem Z* **253**, 42 (1932)
64 Fluhmann C F, and Laqueur, G L *Proc Soc Exptl Biol Med* **54**, 223 (1943)
65 Folley, S J *Biochem J* **30**, 2262 (1936)
66 Folley S J *J Physiol* **93**, 401 (1938)
67 Folley S J *Biol Rev Cambridge Phil Soc* **15**, 421 (1940)
68 Folley S J *Lancet* **240**, 40 (1941)
69 Folley S J *Nature* **150**, 266 (1942)
70 Folley S J and Cowie, A T *Yale J Biol Med* **17**, 67 (1944)
71 Folley, S J, Dyer F J, and Coward, K H *J Endocrinol* **2**, 179 (1940)
72 Folley, S J, and Kon, S K *Nature* **139**, 1107 (1937)
73 Folley S J and Kon, S K *Proc Roy Soc London* **B124**, 476 (1938)
74 Folley, S J and Malpress F H *J Endocrinol* **4**, 1 (1944)
75 Folley S J, and Malpress, F H *ibid* **4**, 23 (1944)
76 Folley, S J, and Malpress, F H *ibid* **4**, 37 (1944)
77 Folley, S J, Malpress, F H, and Young, F G *ibid* **4**, 181 (1945)
78 Folley, S J, Malpress F H, and Young, F G *ibid* **4**, 212 (1945)
79 Folley, S J, Scott Watson, H M, and Amoroso, E C *ibid* **3**, 178 (1942)
80 Folley, S J, Scott Watson H M, and Bottomley, A C *J Physiol* **98**, 15P (1940)
81 Folley, S J, Scott Watson, H M, and Bottomley, A C *ibid* **100**, 7P (1941)
82 Folley S J, Scott Watson, H M, and Bottomley, A C *J Dairy Research* **12**, 1 (1941)
83 Folley, S J, Scott Watson, H M, and Bottomley, A C *ibid* **12**, 241 (1941)

84 Folley, S J, Stewart, D L, and Young, F G *J Endocrinol* **4**, 43 (1944)
85. Folley, S. J, and White, P *Proc Roy Soc London* B120, 346 (1936)
86 Folley, S J, and Young, F G *ibid* B126, 45 (1938)
87 Folley, S J, and Young, F G *Biochem J* **33**, 192 (1939)
88 Folley, S J, and Young, F G *J Endocrinol* **2**, 226 (1940)
89 Folley, S J, and Young, F G *Lancet* **240**, 380 (1941)
90 Folley, S J, and Young, F G *Nature* **148**, 563 (1941)
91 Folley, S J, and Young, F G *J Endocrinol* **4**, 194 (1945)
92. Foss, G L, and Phillips, P *Brit Med J* ii, 887 (1938)
93 Frazier, C N, and Mu J W *Proc Soc Exptl Biol Med* **32**, 997 (1935)
94 Fredrikson, H *Acta Obstet Gynecol Scand* **19**, (suppl 1) 1 (1939)
95 de Fremery, P *Arch néerland zool* **3**, (suppl) 48 (1938)
96 Friedman, M H, and Hall, S R *Endocrinology* **29**, 179 (1941)
97 Gaines, W L *Am J Physiol* **38**, 285 (1915)
98 Gardner, W U, Gomez, E T, and Turner, C W *ibid* **112**, 673 (1935)
99 Gardner, W U, and Turner, C W *Missouri Agr Expt Sta Research Bull* No 158 (1931)
100 Gardner, W U, and Turner, C W *ibid* No 196 (1933)
101 Gardner, W U, and White, A *Proc Soc Exptl Biol Med* **48**, 590 (1941)
102 Gaunt, R. *Am J Physiol* **103**, 494 (1933)
103 Gaunt, R *ibid* **133**, P289 (1941)
104 Gaunt, R *Proc Soc Exptl Biol Med* **47**, 28 (1941)
105 Gaunt, R, Eversole, W J, and Kendall, E C *Endocrinology* **31**, 84 (1942)
106 Gaunt, R, and Tobin, C E *Am J Physiol* **115**, 588 (1936)
107 Gomez, E T *J Dairy Sci* **23**, 537 (1940)
108 Gomez, E T, and Turner, C W *Proc Soc Exptl Biol Med* **34**, 404 (1936)
109 Gomez, E T, and Turner, C W *ibid* **35**, 365 (1936–1937)
110 Gomez, E T, and Turner, C W *ibid* **36**, 78 (1937)
111 Gomez, E T, and Turner, C W *ibid* **36**, 80 (1937)
112 Gomez, E T, and Turner, C W *Missouri Agr Expt Sta Research Bull* No 259 (1937)
113 Gowen, J W, and Tobey, E R. *J Gen Physiol* **15**, 45 (1931)
114 Gowen, J W, and Tobey, E R *ibid* **15**, 67 (1931)
115 Graham, W R, Jr *J Nutrition* **7**, 407 (1934)
116 Graham, W R., Jr *Biochem J* **28**, 1368 (1934)
117 Grattan, J F, and Jensen, H *J Biol Chem* **135**, 511 (1940)
118 Greene, R R *Endocrinology* **29**, 1026 (1941)
119 Griffiths, M *Proc Linnean Soc N S Wales* **67**, 282 (1942)
120 Gruter, F and Stricker, P *Klin Wochschr* **8**, 2322 (1929)
121 Grumbrecht P and von Düsterlho G *ibid* **16**, 513 (1937)
122 Hall, S R *Endocrinology* **34**, 1 (1944)
123 Hall, S R *ibid* **34**, 14 (1944)
124 Hall, S R, and Nicolet B H *J Dairy Sci* **25**, 703 (1942)
125 Hammond, J *Quart J Exptl Physiol* **6**, 311 (1913)
126 Hammond, J *Vet Record* **16**, 519 (1936)
127 Hammond, J, Jr, and Day, F T *J Endocrinol* **4**, 53 (1944)
128 Hathaway, I L, Davis, H P, Reece R P, and Bartlett, J W *Proc Soc Exptl Biol Med* **40**, 214 (1939)
129 Herman, H A, Graham, W R, Jr, and Turner, C W *Missouri Agr Expt Sta Research Bull No* 275 (1938)

130 Herold, L *Arch Gynäkol* **168**, 534 (1939)
131 Himsworth, H P, and McNair Scott, D B *J Physiol* **92**, 183 (1938)
132 Hisaw, F L *Yale J Biol Med* **17**, 119 (1944)
133 Holst, S, and Turner, C W *Proc Soc Exptl Biol Med* **42**, 479 (1939)
134 Houssay, B A *Compt rend soc biol* **120**, 496 (1935)
135 Huffman, J W *Quart Bull Northwestern Univ Med School* **15**, 270 (1941)
136 Hurst, V, Meites, J, and Turner, C W *Proc Soc Exptl Biol Med* **49**, 592 (1942)
137 Hurst, V, Reece, R P, and Bartlett, J W *J Dairy Sci* **23**, 536 (1940)
138 Hurst, V, and Turner, C W *Endocrinology* **31**, 334 (1942)
139 Jack, E L, and Bechdel, S I *J Dairy Sci* **18**, 195 (1935)
140 Jensen, H, and Grattan, J F *Am J Physiol* **128**, 270 (1939–1940)
141 de Jongh, S E *Acta Brevia Néerland Physiol* **3**, 52 (1933)
142 de Jongh, S E *ibid* **3**, 88 (1933)
143 Karnofsky, D *Endocrinology* **30**, 234 (1942)
144 Kenny, M, and King, E *Lancet* **237**, 828 (1939)
145 Knodt, C B, and Petersen, W E *J Dairy Sci* **27**, 449 (1944)
146 Kurzrok, R, and O Connell, C P *Endocrinology* **23**, 476 (1938)
147 Lahr, E L, Bates, R W, and Riddle, O *ibid* **32**, 251 (1943)
148 van Landingham, A H, Henderson, H O, and Weakley, C E, Jr *J Dairy Sci* **27**, 385 (1944)
149 Laqueur, G L *Endocrinology* **32**, 81 (1943)
150 Leblond, C P *Compt rend soc biol* **124**, 1062 (1937)
151 Levenstein, I *Anat Record* **67**, 477 (1937)
152 Lewis, A A, and Turner, C W *Proc Am Soc Animal Production* **33**, 63 (1940)
153 Lewis, A A, and Turner C W *J Dairy Sci* **24**, 845 (1941)
154 Lewis, A A, and Turner, C W *Proc Soc Exptl Biol Med* **48**, 439 (1941)
155 Lewis, A A, and Turner, C W *J Dairy Sci* **25**, 895 (1942)
156 Lyon R *Proc Soc Exptl Biol Med* **51**, 156 (1942)
157 Lyons, W R *ibid* **35**, 645 (1936–1937)
158 Lyons, W R *Endocrinology* **28**, 161 (1941)
159 Lyons, W R *Proc Soc Exptl Biol Med* **51**, 308 (1942)
160 Lyons W R, and Catchpole, H R *ibid* **31**, 299 (1933–1934)
161 Lyons, W R, and Catchpole, H R *ibid* **31**, 305 (1933–1934)
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163 Lyons, W R, and McGinty, D A *ibid* **48**, 83 (1941)
164 Lyons, W R, and Page, E *ibid* **32**, 1049 (1935)
165 Lyons, W R, Simpson, M E, and Evans H M *ibid* **48**, 634 (1941)
166 Macchiarulo, O *Ann Ostetr* **58**, 3 (1936)
167 Mackenzie, K *Quart J Exptl Physiol* **4**, 305 (1911)
168 Markowitz, J, and Simpson, W W *Trans Roy Soc Can* V. **19**, 71 (1925)
169 Markowitz, J, and Soskin, S *Am J Physiol* **79**, 553 (1926–1927)
170 Maxwell, A L I, and Rothera, A C H *J Physiol* **49**, 483 (1914–1915)
171 McPhail, M K *Proc Roy Soc London* B**117**, 34 (1935)
172 McPhail M K *ibid* B**117**, 45 (1935)
173 McQueen-Williams, M *Proc Soc Exptl Biol Med* **33**, 406 (1935–1936)
174 McShan, W H, and Turner, C W *ibid* **34**, 50 (1936)
175 Meites, J, Bergman, A J, and Turner, C W *Endocrinology* **28**, 707 (1941)
176 Meites, J, Trentin, J J, and Turner, C W *ibid* **31**, 607 (1942)
177 Meites, J, and Turner, C W *J Clin Endocrinol* **1**, 918 (1941)

178 Meites, J , and Turner, C W *Proc. Soc Exptl Biol Med* **49**, 190 (1942)
179 Meites J , and Turner, C W *ibid* **49**, 193 (1942)
180 Meites J , and Turner, C W *Endocrinology* **30**, 711 (1942)
181 Meites, J , and Turner, C W *ibid* **30**, 719 (1942)
182 Meites, J , and Turner, C W *ibid* **30**, 726 (1942)
183 Meites, J , and Turner, C W *ibid* **31**, 340 (1942)
184 Memorandum in *Quart Bull Health Organization League Nations* **8**, 909 (1939)
185 Miller, K , and Petersen, W E *J Dairy Sci* **24**, 225 (1941)
186 Mixner, J P , Meites, J , and Turner, C W *ibid* **27**, 957 (1944)
187 Mixner, J P , and Turner, C W *Missouri Agr Expt Sta Research Bull* No 378 (1943)
188 Moon, H D *Proc Soc Exptl Biol Med* **35**, 649 (1936–1937)
189 Muckle, C W *Am J Obstet Gynecol* **40**, 133 (1940)
190 Nelson W O *Endocrinology* **18**, 33 (1934)
191 Nelson, W O *ibid* **19**, 187 (1935)
192 Nelson, W O *Proc Soc. Exptl Biol Med* **33**, 222 (1935–1936)
193 Nelson, W O *Physiol Revs* **16**, 488 (1936)
194 Nelson, W O *Am J Physiol* **126**, P592 (1939)
195 Nelson, W O , and Gaunt, R. *Proc Soc Exptl Biol Med* **34**, 671 (1936)
196 Nelson, W O , and Gaunt, R *ibid* **36**, 136 (1937)
197 Nelson, W O , Gaunt, R , and Schweizer, M *Endocrinology* **33**, 325 (1943)
198 Nelson, W O , Himwich, H E , and Fazekas, J F *Anat Record* **66**, 201 (1936)
199 Nelson, W O , and Pfiffner, J J *Proc Soc Exptl Biol Med* **28**, 1 (1930–1931)
200 Nelson, W O , and Pfiffner, J J *Anat Record* **51**, 51 (1931 1932)
201 Nelson, W O and Pichette J W *Federation Proc* **2**, 36 (1943)
202 Nelson, W O , and Smelser, G K *Am J Physiol* **103**, 374 (1933)
203 Nelson, W O , and Tobin, C E *Endocrinology* **21**, 670 (1937)
204 Newton, W H , and Richardson, K C *J Endocrinol* **2**, 322 (1941)
205 Noble, R L *ibid* **1**, 128 (1939)
206 Ott, I , and Scott, J C *Proc Soc Exptl Biol Med* **8**, 48 (1911)
207 Parkes, A S , and Bellerby, C W *J Physiol* **62**, 301 (1926–1927)
208 Parkes, A S , and Glover, R E *J Endocrinol* **4**, 90 (1944)
209 Pencharz, R I , and Long, J A *Am J Anat* **53**, 117 (1933)
210 Pencharz, R I , and Lyons, W R *Proc Soc Exptl Biol Med* **31**, 1131 (1933–1934)
211 Pencharz, R I , and Lyons, W R *ibid* **38**, 388 (1938)
212 Petersen, W E *J Dairy Sci* **25**, 71 (1942)
213 Petersen, W E *Proc Soc Exptl Biol Med* **50**, 298 (1942)
214 Petersen, W E *Physiol Revs* **24**, 340 (1944)
215 Petersen, W E , and Ludwick, T M *Federation Proc* **1**, 66 (1942)
216 Preheim, D V *Endocrinology* **27**, 494 (1940)
217 Ralston, N P , Cowsert, W C , Ragsdale, A C , Herman, H A , and Turner, C W *Missouri Agr Expt Sta Research Bull* No 317 (1940)
218 Ramos, A P , and Colombo, E *Deut med Wochschr* **64**, 782 (1938)
219 Reece, R P *Proc Soc Exptl Biol Med* **39**, 77 (1938)
220 Reece, R P *ibid* **40**, 25 (1939)
221 Reece, R P *ibid* **42**, 54 (1939)
222 Reece, R P *ibid* **46**, 265 (1941)
223 Reece, R. P *J Dairy Sci* **24**, 497 (1941)
224 Reece, R P *Proc Soc Exptl Biol Med* **52**, 145 (1943)

225 Reece, R P *J Dairy Sci* **27**, 545 (1944)
226 Reece, R P, Bartlett, J W, Hathaway, I L, and Davis, H P *Proc Soc Exptl Biol Med* **43**, 183 (1940)
227 Reece, R P, and Bivins, J A *ibid* **49**, 582 (1942)
228 Reece, R P, Hathaway, I L, and Davis, H P *J Dairy Sci* **22**, 1 (1939)
229 Reece, R P, and Mixner, J P *Proc Soc Exptl Biol Med* **40**, 66 (1939)
230 Reece, R P, and Turner, C W *ibid* **35**, 367 (1936–1937)
231 Reece, R P, and Turner, C W *ibid* **35**, 621 (1936–1937)
232 Reece, R P, and Turner, C W *Missouri Agr Expt Sta Research Bull* No **266** (1937)
233 Reineke, E P, Bergman, A J, and Turner, C W *Endocrinology* **29**, 306 (1941)
234 Reineke, E P, and Turner, C W *J Dairy Sci* **25**, 393 (1942)
235 Riddle, O, and Bates, R W Sex and Internal Secretions, 2nd ed Baillière, Tindall and Co London, (1939), p 1088
236 Riddle, O, Bates, R W, and Dykshorn, S W *Proc Soc Exptl Biol Med* **29** 1211 (1931–1932)
237 Riddle, O, Bates, R W, and Dykshorn, S W *Am J Physiol* **105**, 191 (1933)
238 Riddle, O and Braucher, P F *ibid* **97**, 617 (1931)
239 Robson, J M *Quart J Exptl Physiol* **24**, 337 (1935)
240 Robson, J M *Proc Soc Exptl Biol Med* **36**, 153 (1937)
241 Ross, J R *Endocrinology* **22**, 429 (1938)
242 Rowlands, I W *J Endocrinol* **3**, 203 (1942)
243 Russell J A., and Craig, J M *Proc Soc Exptl Biol Med* **39**, 59 (1938)
244 Schafer, E A *Quart J Exptl Physiol* **6**, 17 (1913)
245 Schafer, E A *ibid* **8**, 379 (1914–1915)
246 Schafer, E A, and Mackenzie, K *Proc Roy Soc London* **B84**, 16 (1911–1912)
247 Scharf, G, and Lyons W R *Proc Soc Exptl Biol Med* **48**, 86 (1941)
248 Schooley, J P, and Riddle, O *Am J Anat* **62**, 313 (1938)
249 Schultze, A B, and Turner, C W *J Dairy Sci* **16**, 129 (1933)
250 Selye, H *Proc Soc Exptl Biol Med* **31**, 488 (1933–1934)
251 Selye, H *Am J Physiol* **107**, 535 (1934)
252 Selye H, Collip, J B, and Thomson, D L *Proc Soc Exptl Biol Med* **30**, 588 (1932–1933)
253 Selye, H, Collip, J B, and Thomson, D L *ibid* **30**, 589 (1932–1933)
254 Selye, H, Collip, J B, and Thomson, D L *ibid* **31**, 82 (1933–1934)
255 Selye, H, Collip, J B, and Thomson, D L *Endocrinology* **18**, 237 (1934)
256 Selye H, and McKeown, T *Anat Record* **60**, 323 (1934)
257 Shaw, J C *J Dairy Sci* **25**, 1051 (1942)
258 Smith, G van S, and Smith, O W *Am J Physiol* **103**, 356 (1933)
259 Smith J A B *J Dairy Research* **14**, 195 (1945)
260 Smith, J A B, and Dastur, N N *Biochem J* **34**, 1093 (1940)
261 Smith, P E *Am J Physiol* **99**, 345 (1931–1932)
262 Spielman, A, Ludwick, L M, and Petersen, W E *J Dairy Sci* **24**, 499 (1941)
263 Spielman, A A, Petersen, W E, and Fitch J B *ibid* **27**, 441 (1944)
264 Spoor H J, Hartman, F A, and Brownell, K A *Am J Physiol* **134**, 12 (1941)
265 Spriggs, D N *Vet Record* **57**, 519 (1945)
266 Stewart, H L., Jr, and Pratt, J P *Endocrinology* **25**, 347 (1939)

267 Stockklausner, F , and Daum, F *Milchw Forsch* **13**, 448 (1932)
268 Stricker P and Grueter F *Compt rend soc biol* **99** 1978 (1928)
269 Swanson, E W , and Turner, C W *J Dairy Sci* **24**, 635 (1941)
270 Swingle, W W , and Pfiffner, J J *Medicine* **11**, 371 (1932)
271 Sydnor, K L. *Endocrinology* **36**, 88 (1945)
272 Sykes, J F , Gould, I A , Duncan, C W , and Huffman, C F *J Dairy Sci* **27**, 539 (1944)
273 Sykes, J F , Meuleman, W L , and Huffman, C F *Endocrinology* **30**, 217 (1942)
274 Tgetgel, B *Schweiz Arch Tierheilk* **68**, 335 (1926)
275 Tgetgel, B *ibid* **68**, 369 (1926)
276 Tobin, C E *Proc Soc. Exptl Biol Med* **41**, 599 (1939)
277 Trautmann, A , and Kirchhof, H, *Deut tierärztl Wochschr* **47**, 654 (1939)
278 Turner, C W , and Cooper, W D *Endocrinology* **29**, 320 (1941)
279 Turner, C W , and Cupps P T *ibid* **24**, 650 (1939)
280 Turner, C W , and Meites J *Proc Soc Exptl Biol Med* **47**, 232 (1941)
281 Turner, C W , and Meites J *Endocrinology* **29**, 165 (1941)
282 Turner, C W , and Slaughter, I S *J Dairy Sci* **13**, 8 (1930)
283 Vogt, M *J Physiol* **103**, 317 (1944–1945)
284 Walker, S M , and Stanley A J *Anat Record* **78** (suppl), 142 (1940)
285 Walker, S M , and Stanley, A J *Proc. Soc Exptl Biol Med* **48**, 50 (1941)
286 Walsh, J W , and Stromme, W B *Am J Obstet Gynecol* **47**, 605 (1944)
287 Waterman L., Freud, J , and Vos-de-Jongh, N *Acta Brevia Neerland Physiol* **6**, 84 (1936)
288 Weichert, C K , and Kerrigan, S *Endocrinology* **30**, 741 (1942)
289 Werner, A A *ibid* **24**, 119 (1939)
290 Winson, S G *Am J Obstet Gynecol* **46**, 545 (1943)
291 Young, F G *Biochem. J* **32**, 1521 (1938)

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